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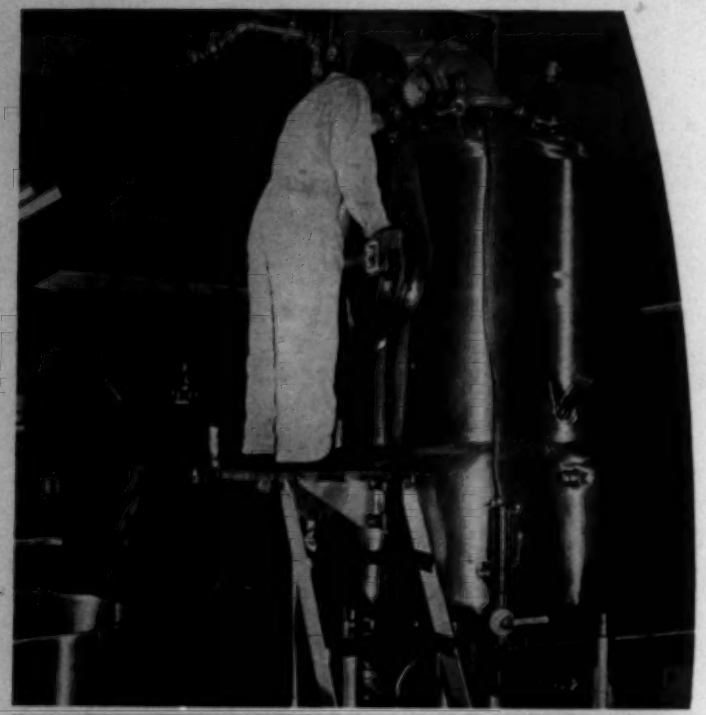

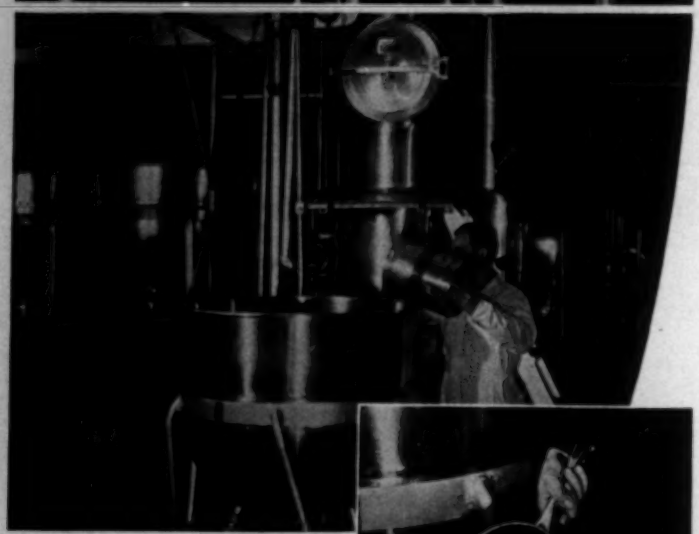

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Food Wastage — A Technology Problem

The problem of food supply is one that involves production and utilization. It should be understood that our food standard is one of the highest in the world and every move should emphasize the need to raise the level in those foods that furnish satisfaction and good nutrition. The problem of food supply is not one that is immeditely pressing in this country. Better processing, preservation, and distribution to prevent loss of nutrients as well as actual loss of food is of equal importance to increased production in keeping a balance between food supply and increased population.

The time will come when our present rate of food wastage due to spoilage in the field, in storage and distribution channels, and in the kitchen cannot be tolerated even in our own country without lowering our standards seriously. What percentage of our supply is wasted is unknown. It is sufficiently large however to offer ample reward for technological developments that will improve conservation. Such conservation may be effected in many ways that are not ordinarily classed as preservation. More convenient packaging should discourage waste in the home. Better storage and better processing may avoid waste of nutrients through deterioration. New developments in harvesting methods will certainly avoid large field losses. These savings are of equal importance with increased production in our race to feed a rapidly growing population.

The world has been slow in solving its food problems, but, for that matter, it has been slow in solving its transportation, communication, clothing, and housing problems. The difference is that people have always recognized food shortage while they had no conception of airplanes, telephones, nylon, and insulated houses, until these things were developed and handed to them. Therefore, one might have expected more attention to the food situation at an earlier date. The plain fact is that developments in all of these fields could not possibly have taken place until man had perfected the experimental method of acquiring accurate information about himself and his environment.

If developments in food have appeared to be somewhat less spectacular than in electronics, let us remember that the human body which utilizes this food is a most intricate system of balanced and coordinated chemical reactions with a fixed relationship. Since we cannot change the machine which uses the food, we must always have products that possess the nutrients in a form not greatly different than that eaten by our ancestors a hundred thousand years ago. While we know much more about the source, the chemical composition, and the function of these nutrients, we have not been able to make any important substitutions. Except for a few of the vitamins which have been synthesized, it may be said that we are dependent today on the same natural sources for our food as were our earliest predecessors. The boundaries that circumscribe the efforts of food scientists are likely to remain until some millions of years may allow the evolution of man into a different physiological specimen.

While the limitation may thwart the ambitious promoter who would like to be the first to provide the astonished world with a square meal in a package no larger than an aspirin tablet, it should also serve as an important guide to the more practical food technologist. Our field is now clearly marked as production, processing, preservation, packaging and distribution of the many long recognized food items in such a manner as to provide the consumer with enough of all the nutrients in palatable form, convenient to use and free of contamination. Meeting these requirements for a world population under the widest possible variation of environment and with each variant under continuous change, but with each consumer a rigidly fixed physiological unit, should be sufficient challenge. These are the problems of the food techcnologist.

R. C. Newton
I. F. T. President 1942-43

New or Old White House?

Since President Truman, in his inaugural address, expressed the idea that "We must embark upon a bold new program of making the benefits of our scientific advances and industrial progress available for the improvement and growth of underdeveloped areas," there has been much discussion of two questions; first, to what extent are we qualified to benefit the underdeveloped areas by transmitting to them knowledge of our scientific advances and industrial progress? second, what accounts for the fact that we are able to show the way technologically to other nations, if we are so qualified?

Many comparisons are being made. A favorite of these involves Great Britain and the United States, although, in such a comparison, we neglect the "underdeveloped areas." In this comparison, we learn that, for research, Britain spends 0.9 percent of its national income, and United States spends 0.6 percent of its national income. These expenditures amount to $6.75 per capita in the United Kingdom and $7.95 per capita in the United States. Since $6.75 will pay for more research in Great Britain than will $7.95 in the United States, it appears that research is at least more intense in Great Britain than in the United States. One may state that results seem to reflect the logical expectation from that situation—that Great Britain leads the United States in the production of fundamental scientific knowledge.

In admitting this fact, we have not answered the question of to what extent the United States is qualified to help other nations technologically. Certainly, if the United States is qualified to help Great Britain, one of the world's most advanced nations, it is more than qualified to help a "backward" nation, as Mr. Truman suggests. To answer the question, we may simply say that it is generally admitted that technology in the United States is ahead of technology in any other country. That seems to be a fairly satisfactory answer to question No. 1.

The second question is, why does this situation exist? Perhaps Americans are more inclined to convert scientific knowledge into practical use because they have greater appreciation of the pleasures originating in the use of new things than do the people of other nations. Could any thinking person who has observed the love of tradition existing in peoples of the Old World fail to believe that their adoration of the things of the past could fail to muffle desires on the part of those peoples to desert the material things of the past in favor of new ones?

Americans, thus far, have been inclined to restrict to historical considerations the sentiment that compels the preservation of material things that are "steeped in tradition." We have been willing to consign to the museum material things of a useful nature which bear sentimental attachments, as soon as they have lost their economic value. If such inclinations are to continue to determine our course of action, we shall have to guard against our losing the sense of distinction between preserving things which are to be used for the purpose of educating us or of satisfying our sentimentality and preserving things in substance which must continue to serve purposes that have direct economic significance.

It is reasonable that sentiment should prevail in the consideration of things of the former type. In connection with things of the latter type, however, the usefulness factor must overrule the factor of sentiment; at the same time, both sentimental value and usefulness can often be preserved together. This is particularly true in the case of buildings. Does not the atmosphere created by a building rather than the specific mortar and stones of which the building is made establish the sentimental value which is attached to that building? The atmosphere can be preserved within walls which are new from top to bottom. Numerous examples, even in the United States, prove this.

We Americans may be approaching the point in the building of our traditions at which we will begin to become confused as to what is the true relationship between sentimental attachment and usefulness. If so, we shall have to begin to look to our laurels in the field of technology. While confusion of this sort may appear to be far removed from the realm of possibility in the field of food technology, even food technologists cannot afford to be complacent; they are not protected from habits of thought by an iron curtain. Moreover, to the extent that food technologists in America may not be as susceptible to the influence of tradition as are technologists in some other lines, food technologists of other countries probably are similarly removed from such influence.

C. Olin Ball.

Notes and Letters

Increasing Colony-Medium Contrast for Improved Plate Counting

The unstandardized nature of the "standard plate count methods" has been demonstrated often by the greatly different numbers reported as the bacterial count of a sample by different laboratories or even by different workers in the same laboratory. A difference in visual powers may account for some of this variation, even when a magnifying plate-counter is used.

The modification described below tends to minimize variation in visual powers as a factor in varied plate counts by increasing the contrast between medium and colonies. The procedure, adaptable to almost all plating methods and particularly to the analysis of dairy products, is as follows:

Incorporate 0.1-0.2% soluble starch in the plating medium. Follow usual procedures of diluting, plating and incubation. When the plates are ready to be counted, flood the plate briefly with a dilute aqueous iodine solution (Lugol's or Gram's Iodine Solution), then pour it off. The iodine may be poured from one plate to another. Count colonies using transmitted light. The iodine reacts with the starch in the medium to give the medium a royal blue color. With this background the colonies stand out brightly and without color other than the pigment they may possess. The overall intensity of light through the plate is reduced greatly; at the same time the contrast between medium and colonies is improved so greatly that even pinpoint colonies show up clearly. Surface and subsurface colonies show up equally well. A colony of an organism with diastatic powers is surrounded by a clear colorless zone. While this is of no particular advantage in total counting it is not detrimental.

The use of the method proposed above for plate counting appears to lessen greatly the factor of eye fatigue when numerous plates are to be counted within a short period of time.

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Gelation Characteristics of Acid-Precipitated Pectinates[a]

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Pilot-plant production of low-methoxyl pectin from citrus peel by means of a counter-current system, and subsequent alkaline de-esterification and acid precipitation is briefly described. Evidence is presented relative to the differences in structures between low-methoxyl, low-solids and high-methoxyl, high-solids gels of pectinic acid. Place of low-methoxyl pectin in the food field is pointed out.

Method of Production

A study of low-methoxyl pectin, its production, uses, and properties, has been under way at the Western Regional Research Laboratory for a number of years. The project was instituted because of its possible contribution to the better utilization of some of the millions of pounds of peel and pomace from citrus and apple processing operations. This paper describes briefly the process under development for the production of low-methoxyl pectinic acids and discusses their gelling properties.

Three generally applicable methods of de-esterification have been discussed in this Symposium. Research at the Western Regional Research Laboratory has stressed the use of alkali as the de-esterifying agent because of the ease of control and adaptability to continuous operation. Results have been sufficiently promising to warrant experiments on pilot-plant scale.

De-esterification is only one phase of low-methoxyl pectin production that has required examination; consequently the pilot plant evolved covers the entire operation from preparation of citrus peel for extraction to final drying of isolated pectinate. Apparatus used is pictured in Figure 1 and is capable of handling about 400 pounds of waste peel per hour. The flow sheet is shown in Figure 2.

Blanched, sliced citrus peel is given a warm-water leach to remove sugars, some colloidal material, and other water-soluble impurities. The leached peel is dried to provide for year-round operation. A continuous counter-current extractor shown in Figure 3 resulted from extensive experimentation and was designed by L. J. Graham and A. D. Shepherd of this Laboratory. Peel is fed into the lower end and is moved through the extraction liquor by means of a screw conveyor, which gives continuous but gentle agitation, decreasing the extent of disintegration of peel and also the amount of fine particles, as compared with batch extraction. The liquor removed from the overflow is relatively clear and easily filtered. Extracted peel is scraped over a screen, washed, and dumped. Conditions presently used for extraction are a water-to-fresh-peel ratio of 3 to 1 (for dry peel about 30 to 1), incoming extraction liquor adjusted to pH 1.3 to 1.4, a temperature of between 194° and 212° F. (90° and 100° C.), and a duration of about one hour.

[a] Presented before the Eighth Annual I. F. T. Convention, Philadelphia, Pa., June 8, 1948.

[b] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

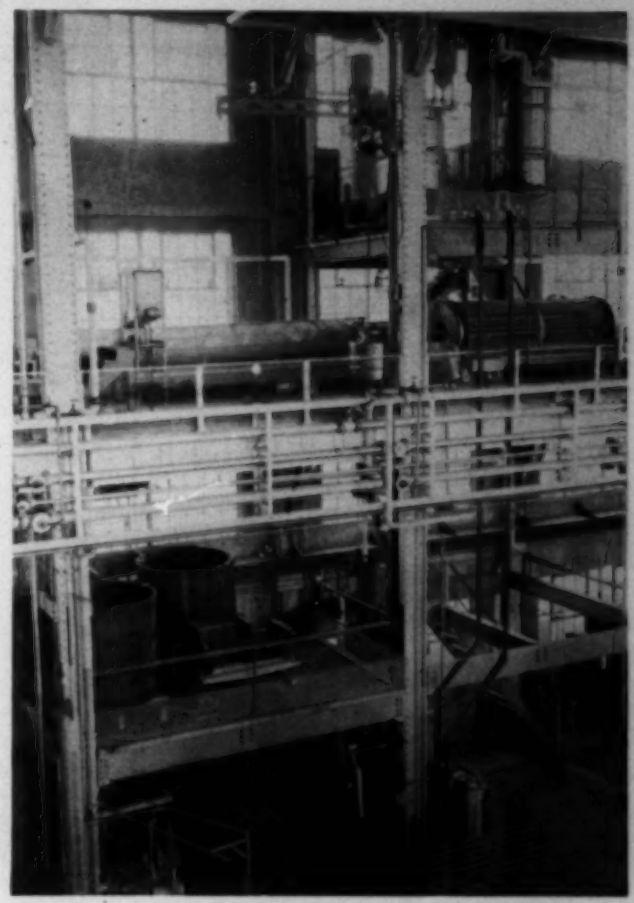

FIG. 1. View of pilot plant for production of low-methoxyl pectin at the Western Regional Research Laboratory. Extractor at third level, filter press on second, vacuum evaporative cooler and de-esterification tanks on fourth, and reel and press on third.

The liquor is passed through an air-evaporative cooler where its temperature is lowered to 95° to 104° F. (35° to 40° C.). It is then filtered and stored. Vacuum evaporation is used to cool the solution to about 55° F. (13° C.). Added ammonia raises the pH to 10.5 and de-esterification proceeds for about 2.5 hours *(7)*. Sulfuric acid is then added to lower the pH to near 1.2, thus stopping the de-esterification and precipitating the pectinic acid *(8)*. The resulting weak gel flows into a dewatering reel, is pressed, washed, and dewatered again to about 35 percent solids. Sodium bicarbonate is milled in to about half-neutralization, and the material is air-dried. The final product has a methoxyl content of about 3.5 percent and an intrinsic viscosity above 3 on an ash- and moisture-free basis and is obtained in the same yield as in the batch process.

Experimental

The pectins used in this investigation are described in Table 1. They or similar products have been used in previous investigations *(9, 10, 11, 13, 14, 16)*, and considerable information on their properties is available.

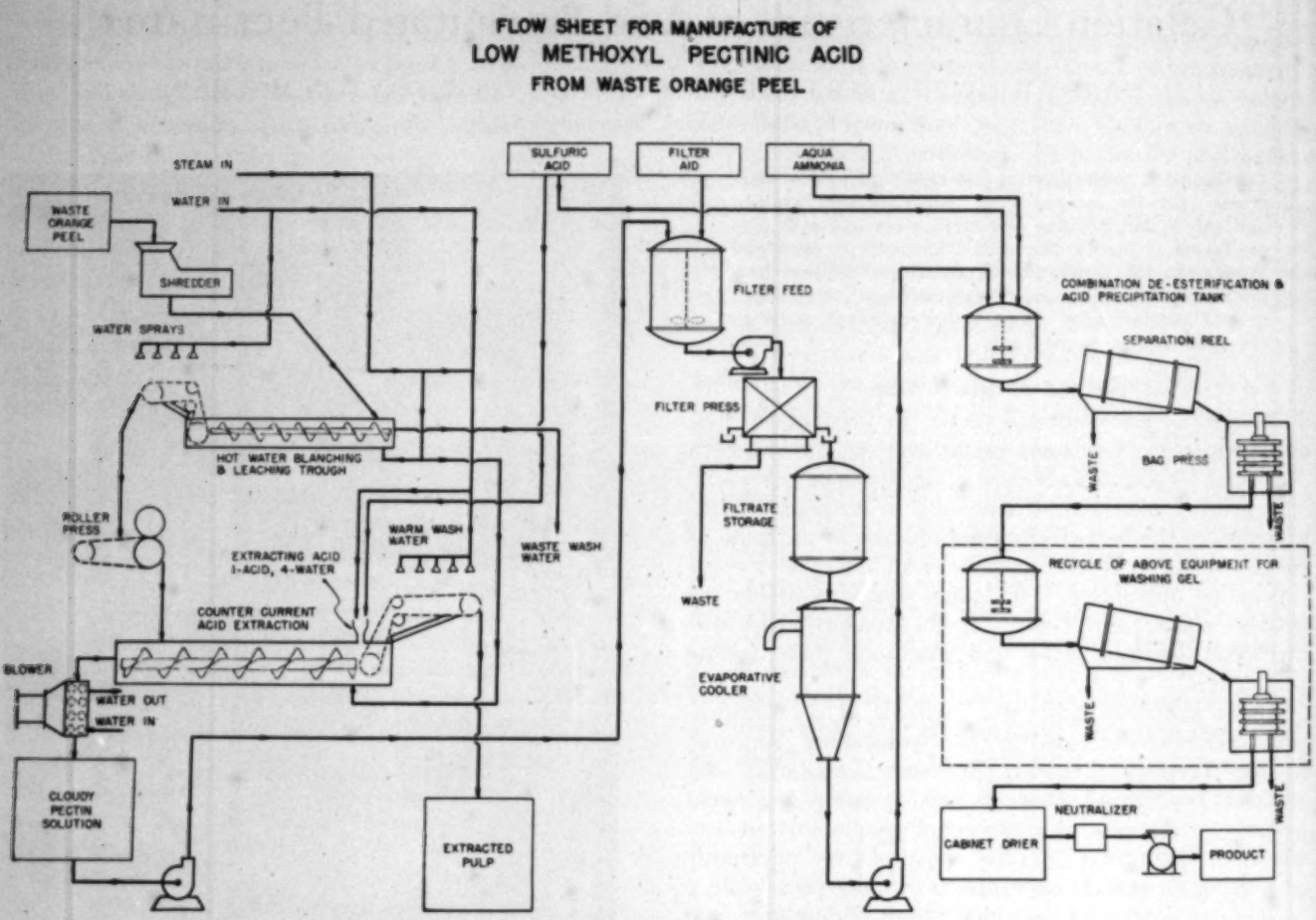

FIG. 2. Flow sheet for production of low-methoxyl pectin.

TABLE 1

Description of Pectinic Acids Used in This Investigation

Sample No. as MeO in %	[η]	Ash %
10.1	4.2	0.6
7.4B *	3.5	0.5
5.3B	3.4	0.5
4.9B	3.3	0.5
4.5B	3.3	0.5
3.5B	3.1	0.5
0.0B	1.9	0.3
Commercial sample of citrus pectinate 4.2	3.6	1.0
Commercial sample of apple pectinate 5.0	4.1	14.2

* B = Alkaline de-esterification.

Methods of preparing the high-solids gels and of measuring the amount of sag by the Cox and Higby tester *(4)*, the breaking strength by the Tarr-Baker tester, and the shear modulus by the rigidometer have been described in detail *(11)*. The low-solids gels are prepared in the following way: for 750 g. of gel, 7.5 g. of pectinate and 80 g. of sugar are added to 300 g. of water containing 0 to 15 ml. of 2*N* sodium citrate and the mixture is heated for one minute. The rest of the water, containing the required amount of calcium chloride and 0 to 15 ml. of 2*N* citric acid with the rest of the sugar, is heated to boiling. The two solutions are mixed and boiled for an additional two minutes. The quantity of water is adjusted so that only a small amount of additional water or additional boiling gives the desired final weight. The gelling solution is then poured into jelly glasses with sideboards and allowed to cool at room temperature (about 73° F., 23° C.) for at least 16 hours. The top of the gel is sliced off and measurements are usually made with the Tarr-Baker instrument, the sagometer, and the rigidometer.

Gelation Characteristics

The properties of acid-precipitated pectinic acids are similar to those isolated by other methods. They are dependent upon the method and degree of de-esterifica-

FIG. 3. Continuous counter-current extractor for extraction of pectin from citrus peel.

tion, and data subsequently reported here are typical of alkali-de-esterified pectinates.

Variables which influence the properties of pectinate gels are concentrations of acid, calcium, pectinate, and sugar, nature of the pectinate, and the temperature. These variables have been studied with both high- and low-methoxyl pectins.

At a given calcium-to-pectinate ratio, decreasing the pH increases the modulus of rigidity of pectinate gels until a pH between 2.5 and 3.0 is reached. The rigidity decreases rapidly below that value, as illustrated in Figure 4. Similar results have been noted in viscosity

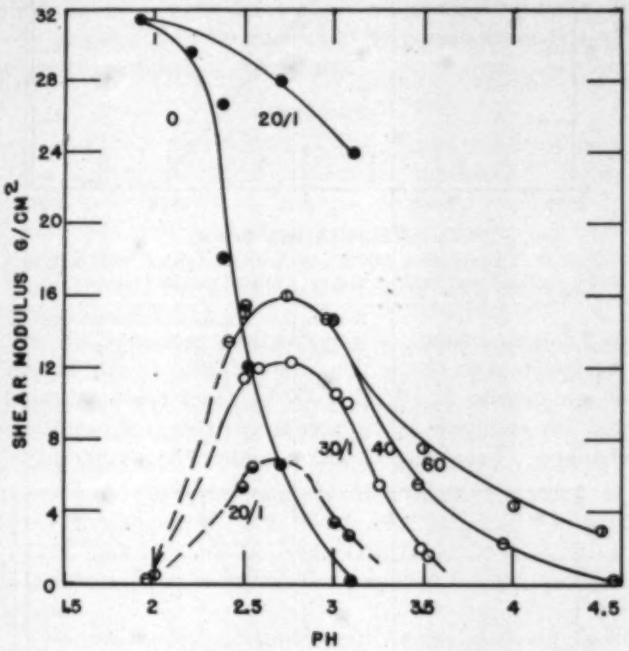

FIG. 4. Influence of pH on shear moduli of low-methoxyl (open-type circles) and high-methoxyl pectin gels (closed circles). Numbers on curves refer to ratio of calcium to pectin in mg./g.

curves of low-methoxyl pectin *(1, 13, 15)*. High-methoxyl pectin, in the absence of polyvalent cations, except the added calcium, and of presetting, fails to yield a maximum in the range of calcium concentrations used. Instead, there is a leveling of shear moduli values to those obtained at pH values below 2.5, as shown in Figure 4 and in a previous publication *(10)*.

Increase in calcium-to-pectinate ratio increases the rigidity of the gel and makes the maximum more pronounced. Curves showing this relationship are included in Figure 4. Maxima in the curves of strength-vs.-calcium concentration have been reported *(5, 6)*. They appear to result from difficulty encountered in obtaining a uniform dispersion when the calcium-to-pectinate ratio is high. If the methoxyl content of the pectinic acid is increased, the calcium-to-pectinate ratio required to produce a gel of a given rigidity must be increased, as illustrated in Figure 5 and as found by previous workers *(1, 5)*.

Changes in sugar concentration markedly influence the rigidity of high-methoxyl pectin gels but have little influence on low-methoxyl pectin gels. This latter statement is true only if the pectin concentration is expressed on the basis of amount of water present instead of on the basis of total weight. Table 2 illustrates the results obtained when the pectin concentration is kept constant at 1 percent of the weight of the water. It may be noted that changing the sugar concentration of low-methoxyl pectinate gels has little influence on rigidity, while no gel is formed with high-methoxyl pectin until the sugar

TABLE 2

Effect of Sugar Concentration on Shear Modulus of Pectinate Gels. Pectin Concentration is 1 Percent of Water Present

Solids %	Specific Gravity	Shear modulus in g./cm.²	
		Low-methoxyl No. 3.5B	High Methoxyl No. 10.1
0	0.998	10.2	0
20	1.08	10.4	0
35	1.15	10.2	0
50	1.23	10 + *	0
60	1.29	.....	5.0
65	1.32	.....	5.8
70	1.35	.....	4 + *

* Irregular gels.

concentration is above 50 percent under the conditions used.

Increasing pectinic acid concentration increases the strength as a logarithmic function, as shown in Figure 6. The difference in slopes may not be significant; the number of experiments is too small to arrive at a definite conclusion.

One of the most marked differences between high- and low-methoxyl pectin gels is in the influence of temperature under investigation by J. C. Miers of this Laboratory. The results are shown in Figure 7. Low-methoxyl pectin gels decrease in rigidity with increase in temperature, while high-methoxyl gels show little change until the temperature is much above 122° F. (50° C.); then they decrease in rigidity as the temperature is further increased.

The last difference to be mentioned is in the curves for deformation of gels vs. stress applied when continuous measurements are made. This has been

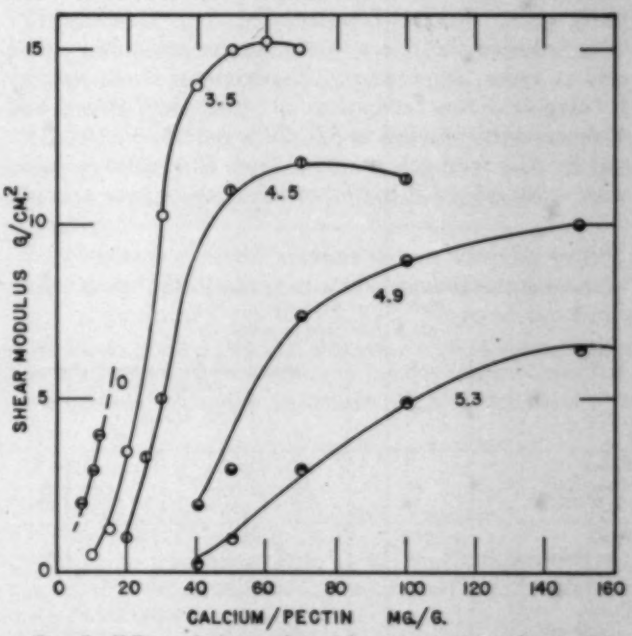

FIG. 5. Effect of change in calcium to pectin ratio on shear moduli of low-methoxyl pectin gels. Numbers on curves refer to methoxyl contents.

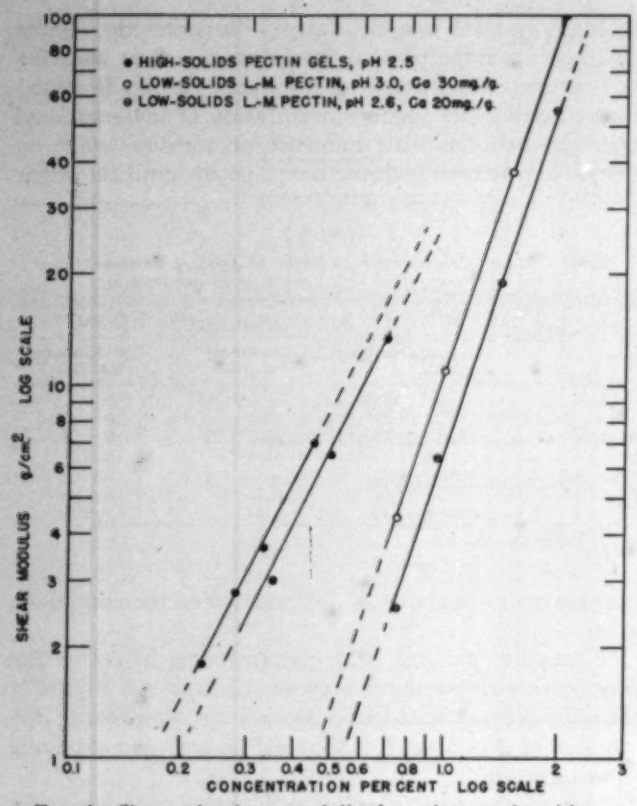
● HIGH-SOLIDS PECTIN GELS, pH 2.5
○ LOW-SOLIDS L.-M. PECTIN, pH 3.0, Ca 30mg./g.
● LOW-SOLIDS L.-M. PECTIN, pH 2.6, Ca 20mg./g.
SHEAR MODULUS g/cm² LOG SCALE
CONCENTRATION PER CENT LOG SCALE

FIG. 6. Change in shear moduli of pectinate gels with concentration of pectinate.

observed by Cheftel and Mocquard *(3)* with a Campbell tester. Similar differences have been observed with plunger-type apparatus and with the rigidometer as shown in Figure 8. High-solids gels appear to become stronger as more force is applied, while low-solids gels show a linear displacement with increasing applied force. In this respect, the former are more like plastic bodies, the latter like elastic ones.

The storage characteristics of low-solids gels are fairly good. Baker, Woodmansee, and Meschter *(2)* have followed the effect of storage on low-solids grape gels at room temperature. Experiments conducted by J. Guggolz of this Laboratory are given in Tables 3 and 4, representing studies at 32°, 77°, and 95° F. (0°, 25°, and 35° C.) with gels prepared from Boysenberry juice, with solids adjusted to 35 percent with sucrose and pH

TABLE 3

Effect of Storage on Shear Modulus of Low-Methoxyl Pectin Gel Made From Boysenberry Juice

pH is 3.2; percent solids, 35

Source	Temp. ° C.	Shear modulus in g./cm.² after storage for No. Months 0	1	3	8
Citrus pectinate (WRRL)	1	3.1	3.6	....	3.6
	25	2.8	4.0	....	4.5
	35	2.0	3.3	3.4	3.5*
Apple pectinate (Comm)	25	3.0	4.2	....	4.7
Citrus pectinate (Comm)	25	2.6	4.0	....	5.5

* Six months.

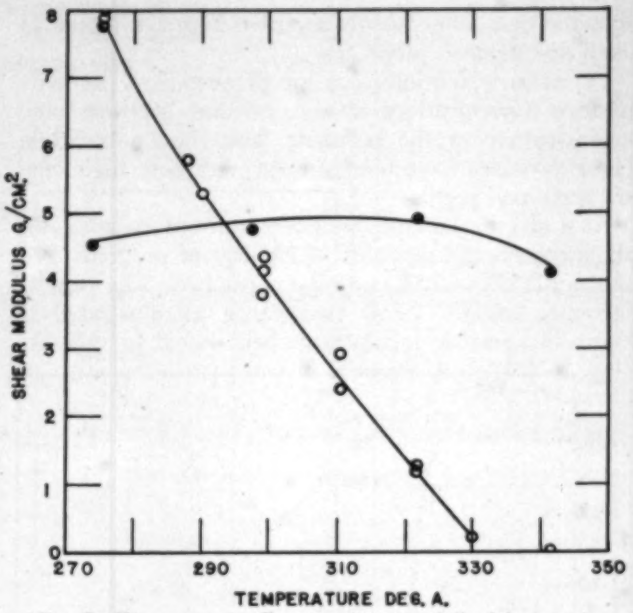

FIG. 7. Temperature effect on shear moduli of low-methoxyl (open circles) and high methoxyl (closed circles) pectin gels.

to 3.2 with citrate. The pectin was recovered by acid precipitation to obtain the data in Table 4. At the end of six months at 95° F. (35° C.) and below, the gel and low-methoxyl pectin recovered from it showed few changes. There were off-colors and off-flavors at 95° F.

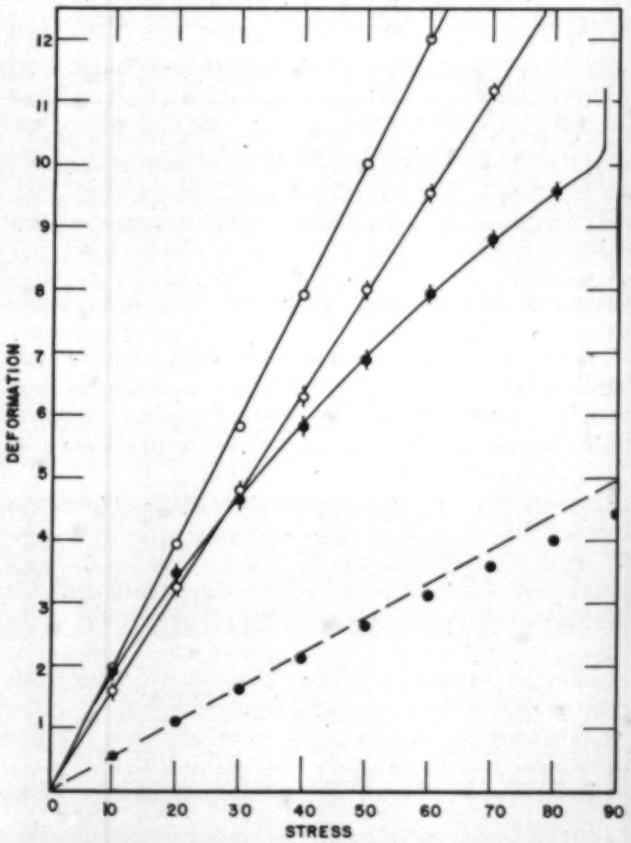

FIG. 8. Deformation of pectin gels versus applied stress. Arbitrary units are used. The applied stress for circles with vertical lines is cm. of CCl_4 in a Tarr-Baker tester. The applied stress for the unlined circles is degrees twist of the torsion head on the rigidometer. Open circles refer to low-methoxyl pectin, closed to high-methoxyl pectin.

TABLE 4

Influence of 35° C. Storage on Properties of Pectinate in Low-Solids Boysenberry Gel

(pH 3.2, total solids 35%)

Time of Storage Months	Pectinate %	Pectinate Methoxyl %	Intrinsic Viscosity dl./g.	Shear Modulus of gel g./cm.²
0	0.95	3.07	2.21	2.0
0.5	1.05	3.55	2.48	3.4
1	1.02	3.31	2.33	3.3
3	1.05	3.56	2.25	3.4
6	0.99	3.63	2.26	3.5

(35° C.), but at 77° F. (25° C.) and 34° F. (1° C.) the gels were satisfactory. There were increases in modulus of rigidity on storage, which may be due to a rearrangement in the molecules to permit formation of a greater number of cross links.

Discussion

Theoretical significance: The results reported here offer further evidence that there are marked differences in the type of bonding between pectinate molecules in high-solids and low-solids gels. Agreement has been general that the former are built up through hydrogen bridges, while the latter are formed through cross bonds involving calcium ion.

Evidence for the former comes from the fact that conditions which favor hydrogen bridging also favor gel formation. For example, in the absence of salts, more rigid gels are formed at pH values where pectin is practically unionized *(12, 16)*. Also, gel formation does not occur until sufficient dehydrating agent is added to desolvate the pectin. This desolvation aids in the formation of hydrogen bridges, as exemplified by association of acetic acid in benzene as compared to lack of it in water.

The presence of calcium bonds in the low-solids gels is hardly questionable. At pH values where pectinic acids are slightly ionized, low-solids gels are greatly weakened. As the methoxyl content of pectins is increased, the solubility of their calcium salts in water increases. Similarly the amount of calcium required to obtain a low-solids gel at methoxyl contents near 5 percent (~ 40 percent esterification) is much higher than when it is near 3 percent (~ 25 percent esterification). The fact that low-solids gels are brittle coincides with expectations for electrovalent links *(14, 15)*. Finally, addition of sugar, when final weights of gel and pectin are kept constant, appears to accomplish little except to increase the concentration of pectinate per unit volume of dispersion and to reduce syneresis. The assumption that pectinate gels are formed through electrovalent bonds supplemented by hydrogen bonds accounts for all of their presently observed properties.

The theory for high-solids gels leaves much to be desired. They appear almost fluid in many respects, yielding to extremely small pressures when they are applied for long periods of time *(3)* [b]. Conversely, they show considerable recovery after deformation resulting from applied force *(15)* [b]. There are several debatable questions still to be answered, such as the presence of sucrose bridges and the presence of carboxyl-to-carboxyl bonds, and much work remains to be done.

[b] Also unpublished data from this Laboratory.

Practical significance: Low-methoxyl pectin should be a valuable new gelling material for the food processor. Gels of required strength can be made under a variety of conditions. For example, canned gelled fruit cocktail and milk pudding present considerable variation in flavor, pH, calcium content, and type of solids, yet both can be readily prepared with low-methoxyl pectin to yield satisfactory products *(7)*.

From the evidence presented, alkali-de-esterified pectinic acids containing less methoxyl than about 2.5 percent may be so sensitive to calcium ion that they cannot be used in fruit juices or even in dessert powders where the water to be added may contain high quantities of calcium salts. At methoxyl contents above 3 percent, little difficulty has been experienced with calcium sensitivity or with syneresis unless the total solids of the gel are reduced to near zero percent.

Food processors may have to give serious consideration to the influence of temperature noted in Figure 7. A product to be shipped through warm parts of the country or to be used at warm temperatures will have to be prepared with more pectin or a higher calcium-to-pectinate ratio than gels destined for use in cooler climates or for use directly from the refrigerator.

Storage life of pectinate gels appears to be as long as that for the flavoring and coloring constituents naturally present in fruit juices. While precise data have been offered for only eight months' storage of Boysenberry gels and 18 months of grape gels *(2)*, some cans of gelled fruit cocktail have been stored for two years and the gel was still in excellent condition, which indicates that the shelf life is sufficient for practical purposes.

Dried pectinate gel formed by dip coating has been found to offer protection against stickiness in candied fruit, dates, and similar food products. Inclusion of such materials as fungicides, antioxidants, and vitamins in the film may bring about many uses for the readily applied coating.

Summary

Pilot-plant production of low-methoxyl pectin from citrus peel through continuous counter-current extraction, alkaline de-esterification, and acid isolation has been briefly described.

Evidence of the differences existing between the structures in low-methoxyl, low-solids and high-methoxyl, high-solids gels of pectinic acids has been presented.

Low-methoxyl pectin appears to be a valuable gelling agent for the food processor. It is compatible with many types of products containing a wide range of type and amount of solids. Storage life of low-methoxyl-pectin gels prepared with Boysenberry juice is longer than that for the flavor and color constituents in the fruit.

LITERATURE CITED

1. Baker, G. L., and Goodwin, M. W. Demethylation of pectin and its effect on jellying properties. Univ. Del. Agric. Expt. Sta. *Bull.* 234 (Oct., 1941).
2. Baker, G. L., Woodmansee, C. W., and Meschter, E. E. "Shelf life" of low-solids grape gels. *Food Technol.*, 1, 11 (1947).
3. Cheftel, H., and Mocquard, J. Contribution to the study of the rheological properties of pectin jellies. *J. Soc. Chem. Ind.*, 66, 297 (1947).

4. Cox, R. E., and Higby, R. M. A better way to determine the jelling power of pectins. *Food Ind.*, **16**, 441 (1944).
5. Hills, C. H., Mottern, H. H., Nutting, G. C., and Speiser, R. Factors influencing the behavior of pectinate gels. Presented before the Am. Chem. Soc., New York City, Sept. 13, 1944.
6. Hills, C. H., White, J. W., and Baker, G. L. Low-sugar jellying pectinates. *Proc. Inst. Food Technol.* **1942**, p. 47.
7. McCready, R. M., Owens, H. S., and Maclay, W. D. Alkali-hydrolyzed pectins are potential industrial products. *Food Ind.*, **16**, 794, 864 (1944).
8. McCready, R. M., Owens, H. S., Shepherd, A. D., and Maclay, W. D. Acidic isolation of low-ester pectinic acids. *Ind. Eng. Chem.*, **38**, 1254 (1946).
9. Owens, H. S., Lotzkar, H., Schultz, T. H., and Maclay, W. D. Shape and size of pectinic acid molecules deduced from viscometric measurements. *J. Am. Chem. Soc.*, **68**, 1628 (1946).
10. Owens, H. S., and Maclay, W. D. Effect of methoxyl content of pectin on the properties of high-solids gels. *J. Colloid Sci.*, **1**, 313 (1946).
11. Owens, H. S., Porter, O., and Maclay, W. D. New device for grading pectins. *Food Ind.*, **19**, 606 (1947).
12. Saverborn, S. A contribution to the knowledge of the acid polyuronides. *Dissertation, Uppsala* (1945).
13. Schultz, T. H., Lotzkar, H., Owens, H. S., and Maclay, W. D. Influence of method of de-esterification on the viscosity and acid behavior of pectinic acid solutions. *J. Phys. Chem.*, **49**, 554 (1945).
14. Schultz, T. H., Owens, H. S., and Maclay, W. D. Pectinate films. *J. Colloid Sci.*, **3**, 53 (1948).
15. Speiser, R., Copley, M. J., and Nutting, G. C. Effect of molecular association and charge distribution on the gelation of pectin. *J. Phys. Colloid. Chem.*, **51**, 117 (1946).
16. Ward, W. H., Swenson, H. A., and Owens, H. S. Electrophoretic mobilities and fractionation of sodium pectinates. *Ibid.*, **51**, 1137 (1947).

High-Polymer, Acid-Demethylated Pectinates and Their Gelation[a]

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The production of high-polymer, acid-demethylated pectinates are considered at 60°C. The speed of demethylation, yields, and characteristics of products are discussed. The dispersion, thickening, and gelation of the calcium-precipitable products in powdered preparations are considered.

The preparation of low-methoxyl pectinates by acid-deesterification has been considered as most satisfactorily carried out below 60° C. *(2)*. No detailed study has been published of the effects of varied time of treatment under conditions of high acidity at 60° C. and above. Since the physical characteristics of pectins have been found to vary with the conditions of demethylation at one temperature *(3)*, it is presumed they may also vary when treated at another. It also is considered quite likely that acid-demethylation can be carried out at temperatures well above 60° C. if properly controlled. The present paper reports an initial phase of study of the effect of high temperature, simultaneous demethylation and extraction of pectins from apple pomace.

The initial phase of study presented at this time considered the effect of variation of the factors, time and pH, of extraction at 60° C. upon yields and the degree of methylation and polymerization, the latter as reflected by grade. It also investigated such characteristics of the products as the ease of precipitation by calcium and acid, and practical uses as thickening and gelling agents in various powdered preparations.

Extraction—Demethylation

Pectic Material. Dried apple pomace from mixed apples was secured from the National Fruit Product Company, Martinsburg, West Virginia. This pomace contained from 9 to 12 percent moisture and is considered representative of average commercial source material for pectin extraction. Prior to use, the pomace was ground to pass a 2 mm. screen in a Wiley Mill.

Conditions of Treatment. The extraction-demethylation of pectinates from apple pomace at 60° C. was carried out at four different levels of acidity (pH 1.0, 0.5, 0.3, and 0.01) with varied periods of treatment. The conditions were adjusted so as to give a wide variation of methoxyl content and grade.

In general, the procedure for preparing the partially demethylated pectinates was as follows:

Five hundred grams of apple pomace (2 mm. particle size) was treated with water containing the specified amount of 38 percent hydrochloric acid at a temperature of 65° C. and the weight of the extracting-demethylating mixture was adjusted to 3000 grams. The resulting temperature was approximately 60° C. The beaker containing the mixture was covered with a cover glass and held in a constant temperature oven at 60° C. for the desired time of treatment. At the end of the treatment the mixture was removed and a small portion used for determining the pH. The mixture was then diluted with 5000 ml. water containing sufficient Na_2CO_3 to adjust the pH of the extract to 4.5. Thorough mixing was necessary. The pectin extract was then separated from the pomace by pressing by hand through a muslin cloth. The relative viscosity (Ostwald at 26° C.) was determined on the extract which was diluted with water, if necessary, to a viscosity between 5 to 8, so as to facilitate later clarification. The pectin extract was clarified, using 30g. of a filter-aid (Standard Super-Cel) per two liters of extract and filtered through a washed pad consisting of filter paper on a 10-inch Büchner funnel covered with 30g. of filter-aid. The clarified pectin extract was thoroughly mixed and tested for starch with iodine. If a slight starch test was noted, sufficient Clarase (Takamin Laboratory, Inc., Clifton, N. J.) was added to hydrolyze the starch. The pectin was then precipitated by adding with agitation an equal volume of 99 percent isoproypl alcohol to the extract. After standing about 20 hours, the pectin was filtered off and pressed by hand using a silk cloth. The pectin was then washed with 75 percent isopropyl alcohol, wrapped with canvas press cloth, and pressed in a Carver press at 10,000 pounds per square inch on the ram. The washing and pressing was repeated. A final washing in 99 percent isopropyl alcohol was carried out. The pectin was dried at 60° C. for 20

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[b] Presented before the Eighth Annual I. F. T. Convention, Philadelphia, Pa., June 8, 1948.

hours, ground to pass a 40-mesh screen, and placed in a closed container for evaluation.

Evaluation of the Pectinates

The detailed conditions of treatment as well as the evaluation of the pectinates extracted as to yield, calcium pectate content, methoxyl content (expressed on the basis of 100 percent calcium pectate) relative viscosity, grade, and optimum pH may be found in Table 1. The data were obtained by employing well-established methods for evaluating partially demethylated pectinates. These methods are described below in the order that they appear in the table.

Yield of Pectinates. The recovery of pectinates, based on the weight of pomace treated, ranged from 5.4 to 11.5 percent.

The data in Table 1 indicate that increases in the acidity of the extraction-demethylation treatment do not materially impair maximum yields and that increases in the time of the extraction period improve the yields to a maximum, after which sharp declines may take place under conditions of very high acidity.

Calcium Pectate. Hinton *(7)* has shown that calcium pectate can be used as a measure of the purity of a pectin. Upon applying his procedure to the partially demethylated pectinates, the calcium pectate content was found to range from approximately 72 to 90 percent.

Methoxyl Content. The methoxyl content was determined by the saponification method of von Fellenberg *(3, 6)* and the results were expressed on the basis of 100 percent calcium pectate for uniformity. Under the conditions of treatment in this study, pectinates were produced which ranged from 3.5 to 9.9 percent methoxyl content.

Figure 1 shows the effect of the time of extraction-demethylation of apple pomace at 60° C. upon the methoxyl content of the prepared pectinates. It shows that at pH 1.0 the rate of demethylation is slow but gradual and easy to control. Even though the demethylation rate is accelerated as the acidity is increased, any desired methoxyl content of a pectin can be obtained easily providing the time, temperature, and degree of acidity are controlled. It is assumed that Figure 1 shows only trends as to the rate of demethylation and that the slopes of the curves may change for various source materials of pectin.

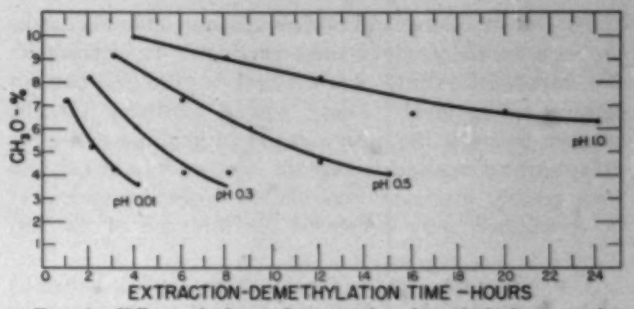

FIG. 1. Effect of time of extraction-demethylation reaction at 60° C. upon the methoxyl content of pectin from apple pomace.

Relative Viscosity. The relative viscosities (Ostwald) were determined upon 0.5 percent solutions of the pectinates at 26° C. at pH 2.5 and pH 4.5. As usual, viscosities of pectinates having a methoxyl content above 7 percent were higher at pH 4.5 than at pH 2.5 although below this methoxyl content the reverse was the case *(3)*. Since increased acidity will increase the viscosity of low-methoxyl pectinates, only viscosities at pH 4.5 are presented in Table 1.

Grade. The grades of these pectinates were determined according to the "normal-boil," pH-jelly strength procedure *(5)* without the addition of buffer salt solution but based on a breaking strength of 50 cm. water pressure using the Delaware Jelly Strength Tester. An assumed grade was first determined from the viscosity measurement *(5)* and it was used to estimate the concentration of pectin solution desired for making the 65 percent soluble-solids test jellies. Since grading from viscosity gives only an approximation, both the high- and low-methoxyl formulas for grade *(1, 3)* were used.

Grades ranging from 68 to 222 were found among these partially demethylated pectinates. In general, the high-acid treatments of the apple pomace must be for short periods and carefully controlled, otherwise the grade of the extracted pectins will be impaired.

TABLE 1

Extraction-Demethylation of Pectin in Apple Pomace at 60° C.

Conditions of treatment			Yield %	Calcium Pectate %	Methoxyl %	Relative Viscosity 0.5% of soln. pH 4.5	Grade	Optimum pH Gelation
38% HCl								
Hours	ml.	pH						
4	50	1.0	5.4	88.03	9.93	4.41	116	2.9
8	50	1.0	6.2	89.63	8.98	6.28	202	2.8
12	50	1.0	7.0	89.65	8.17	6.08	222	2.7
16	50	1.0	8.3	83.55	6.68	5.22	204	2.6
20	50	1.0	8.7	86.20	6.75	4.33	180	2.6
24	50	1.0	8.8	88.53	6.31	4.16	154	2.6
3	100	0.5	6.6	86.25	9.14	7.38	204	3.0
6	100	0.5	7.7	86.35	7.26	5.32	213	2.6
9	100	0.5	8.7	84.65	6.06	3.89	154	2.6
12	100	0.5	9.8	82.30	4.57	3.72	104	2.6
15	100	0.5	11.5	73.80	4.00	2.96	79	2.8
2	200	0.3	7.2	84.25	8.21	6.03	200	2.6
4	200	0.3	8.7	82.75	6.02	3.92	147	2.7
6	200	0.3	10.5	74.55	4.01	2.80	83	2.7
8	200	0.3	9.4	83.23	4.13	3.47	80	2.7
1	400	0.01	8.4	80.43	7.19	4.14	143	2.6
2	400	0.01	10.4	72.13	5.16	2.69	78	2.7
3	400	0.01	10.0	75.75	4.26	2.65	79	2.8
4	400	0.01	5.6	75.10	3.56	2.78	68	2.8

The effect of the addition of calcium and polyphosphates upon the grade of these pectinates was examined. No beneficial effects were found at the 65 percent soluble solids level. There was a tendency for the calcium to lower the optimum pH of gelation while the polyphosphate raised it slightly. An excess of calcium very readily produced coagulation while an excess of polyphosphate only decreased the strength of the test jellies.

The jelly-unit yields (*Grade* times *Yield*, Table 1) when plotted against extraction time as in Figure 2

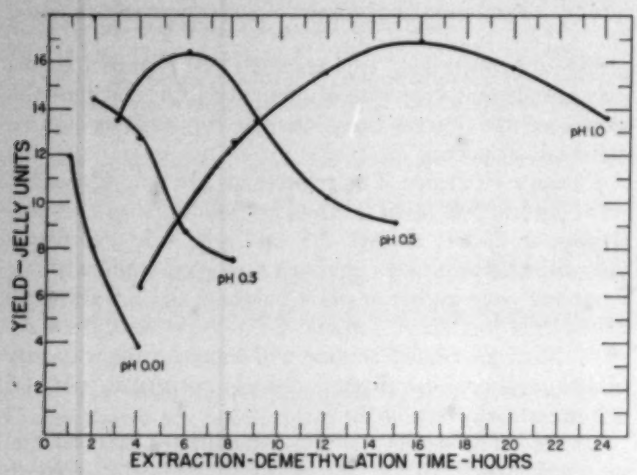

FIG. 2. Variation in jelly-unit yields of pectin with time of treatment.

show that maximums of 16-17 were obtained at both the pH 0.5 and the pH 1.0 levels. Below these pH levels, according to the data, less than two hours extraction-demethylation treatment would be necessary in order to attain maximum jelly-unit yields. The data indicate, however, that yield values might be too low to compensate for higher grade values and the maximum jelly-unit yields obtained at these very high acidities would never reach the maximums found at pH 0.5 to 1.0.

Optimum pH of Gelation. The optimum pH of gelation at the 65 percent soluble-solids level was lowered as the methoxyl contents of the pectinates were reduced in the case of those extracted at a high pH, but the opposite was true for those extracted at the lowest pH. However, the optimum pH of gelation for each of these acid-demethylated pectinates was within a narrow range, pH 2.6 to 3.0.

Discussion

The results of the present preliminary study of the effect of extraction-demethylation of pectins from apple pomace at 60° C. parallel those found at 50° C. *(3)*, except that the rate of demethylation is accelerated due to the higher temperature. The results further indicate that demethylation at the pH 1.0 and 0.5 levels of treatment is gradual and easy to control, while below pH 0.5 a short treatment at 60° C. rapidly reduces methyl ester content and grade without reducing yields. For example, the methoxyl content, grade, and yields of a pectinate prepared in a 4-hour treatment at the pH 0.3 level compares favorably with one from a 9-hour treatment at pH 0.5 or a 24-hour treatment at pH 1.0 (Table 1).

Time has not allowed a full study of the physical characteristics of these pectinates, but several have been investigated. Of possible interest is calcium precipitability. All extracts containing pectinates of less than 8.21 percent methoxyl content were precipitated by calcium chloride. These include all of the pectinates extracted below pH 0.5, all but the first in the series treated at pH 0.5 and all but the first two which were extracted at pH 1.

The degree of polymerization of these pectinates is indicated by their jellying capacity as 65 percent soluble solids gels. Viscosities of their 0.5 percent solutions at pH 4.5 also serve as an index, but a less accurate one. The data indicate maximum grades below those found for pectinates extracted at 50° C. *(3)* and that a rapid decrease in grade, or degree of polymerization, occurs when extraction-demethylation periods are extended at high acidities. Optimum pH of gelation at the 65 percent soluble solids concentration of all pectinates in these groups was below pH 3.

It was indicated from the viscosity measurements at pH 2.5 (not shown in Table 1) that pectins with less than 4 percent methoxyl content could be readily precipitated with acid according to the methods of the Western Regional Research Laboratory *(8)*.

Practical Application

The uses for low-methoxyl pectinates are many and have been well covered in other publications *(3, 4)*. However, as every chef has his own recipes, each partially demethylated pectinate has its own properties. Although one pectinate may be generally similar to another, each has its own characteristics such as response to the addition of the calcium ion, action in gels at different soluble solids levels, etc. With this fact in mind, the authors evaluated these prepared, partially demethylated, pectinates by incorporating them with sugar and calcium salt in powdered preparations and subjecting them to preliminary tests for dispersibility and gelation in the preparation of water gels, milk gels, and grape juice gels. These tests indicate the usefulness of the pectinates in preparing low-solids desserts, salads, etc.

Using a formula of 0.2 gram of pectinate, 2 grams of sucrose (for aid in dispersion of the pectinate), varying increments of monobasic calcium phosphate, and water to make a 20-gram total weight, heat was applied while stirring until the mixture had boiled for 30 seconds. After allowing the preparations to cool, it was found that, below a methoxyl content of 7 percent, satisfactory gels were formed which required on the average about 0.05 gram of the monobasic calcium phosphate. Milk gels were also prepared using the same formula, substituting milk for water. In general, the calcium requirement for the milk gels was lower and the gels were firmer than the water gel from the same pectinate. The grape juice did not gel satisfactorily when the monobasic calcium phosphate was used. Failure appeared to be largely due to improper dispersion of the pectinates. After the pectinates were dissolved, then, upon adding calcium chloride and heating, good grape gels were formed on cooling. Natural fruit salts are known to affect calcium requirements for the gelation of low-methoxyl pectinates, therefore, it is well to make trial

tests in determining amounts. All pectinates which were found to be precipitated with calcium were found satisfactory for some form of low solids gels.

REFERENCES

1. Baker, G. L. How much sugar in fruit jellies? *Food Ind.*, **7**, 170 (1935).
2. Baker, G. L., and Goodwin, M. W. Jellying pectin composition. U. S. Patent No. 2,233,574. (Mar. 4, 1941).
3. Baker, G. L., and Goodwin, M. W. Fruit jellies—XI. The role of pectin—7. Demethylation of pectin and its effect upon jellying properties. Del. Agr. Expt. Sta. *Bull.* **234** (1941).
4. Baker, G. L., and Goodwin, M. W. Fruit jellies—XII. Effect of methyl ester content of pectinates upon gel characteristics at different concentrations of sugar. Del. Agr. Expt. Sta. *Bull.* **246** (1944).
5. Baker, G. L., and Woodmansee, C. W. Fruit jellies—XIII. Grading pectins. Del. Agr. Expt. Sta. *Bull.* **272** (1948).
6. Fellenberg, Th. von. Knowledge of the pectins. *Mitt. Lebens. Hyg.*, **5**, 224-256 (1918).
7. Hinton, C. L. Fruit pectins,—their chemical behavior and jellying properties. Dept. of Sci. and Ind. Res. (British), *Food Invest. Special Report* No. 48 (1939).
8. McCready, R. M., Owens, H. S., Shepherd, A. P., and Maclay, W. D. Acidic isolation of low-ester pectinic acids. *Ind. Eng. Chem.*, **38**, 1254 (1946).

High-Polymer, Ammonia-Demethylated Pectinates and Their Gelation[a]

GLENN H. JOSEPH[b], A. HARRIS KIESER[c], AND EDWIN F. BRYANT[b]

Pectinic acid amides are produced by the carefully controlled treatment of pectin with ammonium hydroxide in alcoholic systems. Chemical and physical properties of this heretofore undescribed type of pectinate are presented. Some commercial applications in the food industry are discussed.

The food industries have become well acquainted, during the past quarter century, with the use of pectin in a dry, powdered form as a valuable ingredient in jellies, jams and confections. During the early days of its commercial development there was only one kind of pectin and its gel-ability or "jelly-grade" was the only criterion of utility. Later there were developed special types of pectin such as: rapid setting, slow setting, emulsifying, confectionery, and then pharmaceutical and medical types.

Scientific knowledge of the structure of pectin necessarily preceded these commercial developments, and consequently the technical publications began using such terms as pectinic acid, pectinates, pectates, and low methoxyl pectin. These various terms are somewhat confusing to most people even at the present time.

The American Chemical Society in 1944 adopted *(8)* a revision of its 1926 nomenclature of the pectic substances. The new and revised definitions use the term "Pectinic Acids" to cover a broad group of pectic substances which "contain" more than a negligible proportion of methyl ester groups. "Pectin" is the term for those pectinic acids which are capable of forming the well-known type of fruit jellies when sugar and acid are present in correct proportions.

The National Formulary, Eighth Edition *(10)*, defines pectin as a "purified carbohydrate product obtained from the dilute acid extract of the inner portion of the rind of citrus fruits, or from apple pomace. It consists chiefly of partially methoxylated polygalacturonic acids." The National Formulary further states that pectin must have more than 7.0 percent methoxyl, on an ash-moisture-free basis. These various definitions have been utilized in preparing the sketch of relationships[d] shown in Figure 1.

The rapidly growing literature on low methoxyl pectinates has been based upon the assumption that the partial de-esterification of pectin may be accomplished by three quite different methods: the use of acids, enzymes or alkalis. It is the purpose of this paper to show that the three methods just listed produce only one general type of product and that another scheme, demethylation with ammonia in alcoholic systems or in concentrated ammonia-aqueous systems, produces a different type, the acid amide. Pectinates made by the three previously used demethylating agents do, however, differ in certain commercially important properties such as viscosity and calcium sensitivity. For this reason it will be best to continue their designation as of three different types and then to add a fourth type as shown in the list below which classifies the products by the agents used in their preparation.

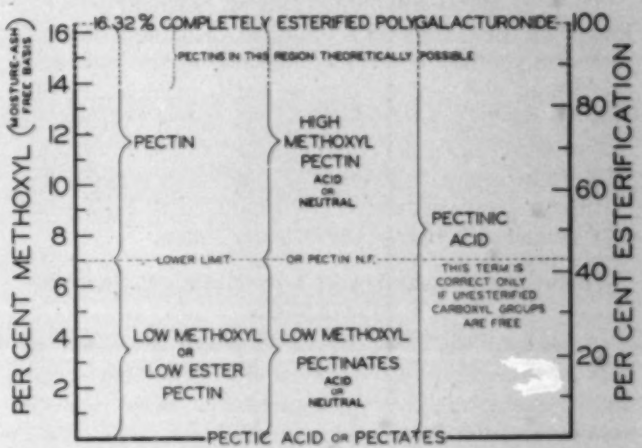
16.32 % COMPLETELY ESTERIFIED POLYGALACTURONIDE
PECTINS IN THIS REGION THEORETICALLY POSSIBLE
PER CENT METHOXYL (MOISTURE-ASH FREE BASIS)
PER CENT ESTERIFICATION
PECTIN
HIGH METHOXYL PECTIN ACID OR NEUTRAL
PECTINIC ACID
LOWER LIMIT OF PECTIN N.F.
THIS TERM IS CORRECT ONLY IF UNESTERIFIED CARBOXYL GROUPS ARE FREE
LOW METHOXYL OR LOW ESTER PECTIN
LOW METHOXYL PECTINATES ACID OR NEUTRAL
PECTIC ACID OR PECTATES

Fig. 1. Nomenclature chart for pectic substances, showing relations between percent methoxyl and theoretical percent of esterification.

1. Acids.
2. Fixed alkalis or dilute aqueous ammonia systems.
3. Enzymes, alone or in connection with alkalis.

[a] Presented before the Eighth Annual I. F. T. Convention, Philadelphia, Pa., June 8, 1948.

[b] Research Department, California Fruit Growers Exchange. Corona, California.

[c] Laboratory, Exchange Lemon Products Company. Corona, California.

[d] The chart of Figure 1 was copied from the 16 page booklet "Pectin L. M." published by the California Fruit Growers Exchange, Ontario, California (1947).

4. Ammonia in alcoholic systems or concentrated aqueous ammonia systems.

The partially de-methoxylated pectins resulting from the first three general processes just mentioned differ considerably from one another in important characteristics. The product prepared by high acid is usually not very sensitive to metallic ions, particularly those of the alkaline-earth elements, while the material produced by enzymes is very sensitive to these metallic ions. This means, in practical usage, that the former pectin requires relatively large amounts of calcium for gel formation and that the pectin made with enzymes is so sensitive to calcium that sometimes the calcium content of hard waters impairs complete solution of the material. Likewise the sols of acid hydrolyzed pectin are very viscous while the enzyme products give more fluid aqueous sols. The ammonia hydrolyzed pectinate (Type 4 above) has a calcium sensitivity and gives a viscosity intermediate between those of the enzyme and the acid types. The fixed alkali demethylated pectinates have a calcium sensitivity between that of the enzyme and ammonia hydrolyzed products. These differences in sensitivity to metallic ions and in viscosity are usually of sufficient magnitude to prevent the different types of pectin from being used interchangeably in any given recipe or formula.

There is yet another difference in behavior which is important in commercial practice. The ammonia de-esterified product is not intended for use in sugar concentrations above 55-60 percent but is particularly suitable for use in gels with low soluble solids (the dessert and salad type of gel as distinguished from the ordinary 65 percent sugar, fruit jelly*). The other types of low methoxyl pectin will give fair results with high solids gels as well as forming gels at lower solids.

Theoretical Discussion of Low Methoxyl Pectinic Acid Amides

Olsen and his associates *(11)* have discussed the theoretical relationship between the combining weight and the percent methoxyl for pectinates, based upon the assumption that the combining weight of pectic acid is 201. The value of 201 agreed with Bonner's previously published *(1)* value and with the hypothesis that two galactose units were involved structurally with each thirteen galacturonic acid units in the pectin molecule. The present authors have frequently produced a pectic nucleus with a combining weight near 180 and Hills and Speiser *(6)* report a value of 178 for one of their samples. It could be considered, therefore, that Schneider's *(14)* contention that pectic acid consists only of chains of galacturonic anhydride units, is correct and that the combining weight of the highly purified pectic nucleus would therefore be 176, the weight of the galacturonic anhydride unit with one carboxyl group. The data of Table 1 were calculated on this assumption.

* The distinction between the words gel and jelly is important to note. The word gel is considered as a general term while jelly applies only to the gels which are commonly known as table jellies and which are defined by the Food and Drug Administration as jellies and jams. These latter jellies are always about 65 percent soluble solids. The soluble solids of gels may be practically zero.

TABLE 1

Theoretical Relations Between Methoxyl, Percent Esterification and Combining Weight, Assuming a Group of Ten Galacturonide Units

Number of Groups		Mol. Wt. n [f]	Combining Weight	Calculated Percent — OCH_3 By Weight	Percent Esterification (Wt. Basis) [g]
— OCH_3	— COOH				
0	10	1760	176	0.00	0.00
1	9	1774	197	1.75	10.72
2	8	1788	224	3.47	21.26
3	7	1802	257	5.16	31.62
4	6	1816	303	6.83	41.85
5	5	1830	366	8.47	51.90
6	4	1844	461	10.09	61.83
7	3	1858	619	11.68	71.57
8	2	1872	936	13.25	81.19
9	1	1886	1886	14.79	90.63
10	0	1900	.....	16.32	100.00

[f] n = one-tenth the actual number of anhydrogalacturonic units in the molecule.

[g] (weight % — OCH_3 ÷ 16.32) 100.

When pectic substances are ordinarily precipitated in the laboratory or are factory made, it is true that the pectic nucleus usually shows a combining weight of about 200 and it is only with difficulty that further purification can be made to produce true pectic acids with a combining weight of near 176. The relationship between percent methoxyl and the combining weights, assuming 176 and 201 for the unit weights, is shown by the two theoretical curves in Figure 2.

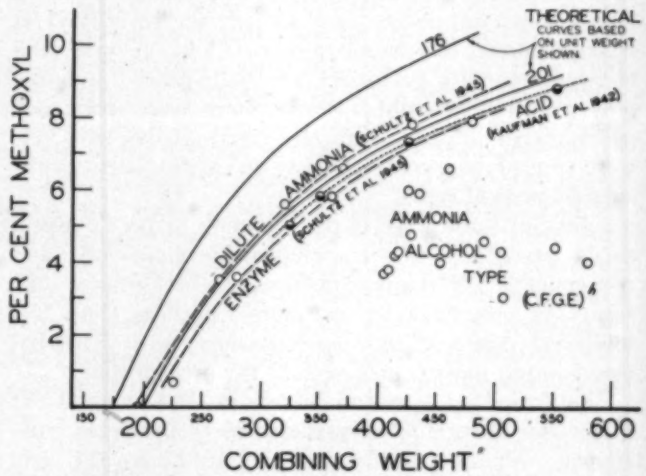

FIG. 2. Relation between combining weight and percent methoxyl for several types of low methoxyl pectin.

[h] C. F. G. E. refers to California Fruit Growers Exchange.

When pectin in alcoholic suspension is subjected to a high concentration of ammonia there is rapid de-esterification and in addition there is a reaction between the ester groups and the ammonia to form the usual type of acid amide. This same reaction can be made to take place in highly concentrated aqueous ammonia systems. Proper control of concentrations, temperatures and times make it possible to retain relatively high average molecular weights and to make a product of 25-35 percent esterification with useful commercial properties.

When the de-esterification has proceeded to the desired extent the system is acidified and the product is alcohol rinsed to free it of the acid and ammonium salt of the acid. Some of the carboxyl groups in the final product will be in combination as ammonium salts, some will be free, others as acid amides and a very few may

exist as metal salts. This production of acid amides is not to be confused with Carson's *(2)* preparation of polygalacturonide n-alkylamides, with Deuel and Neukom's *(4)* scheme of using aliphatic diamines on totally esterified glycol esters of pectic acid, nor with the formation of amine salts from pectic acid as described by Stuewer and Olsen *(17)*.

Analytical Procedures Peculiar to the Ammonia Demethylated Pectinates

The presence of ammonium salts and acid amide groups in pectinates complicates the processes ordinarily used for characterizing these materials. Some of the special technics which we use in studying these particular pectinates are described briefly below.

Methoxyl Determination. The method used for methoxyl determination is based upon the Romeo modification *(12)* of Fellenberg's scheme *(5)*. Samples of 0.500 gram in 100 ml. of water are titrated. The method also has been adapted to a rapid assay of acid-alcohol rinsed pectins as described in the National Formulary, Eighth Edition *(10)*.

Ronchése Method for Ammonium Salts. There are two ways one may determine the distribution of total ammonia between ammonium salts and amide groups. The sample may be acid-alcohol rinsed to remove the salts then distilled with sodium hydroxide to determine the amide ammonia. It is also possible to add neutral formaldehyde to convert the ammonium salts to hexamethylene tetramine then titrate the liberated carboxyls as representing ones originally linked as ammonium salts and then deducting this value from that found for the sodium hydroxide distillation. This use of the Ronchése titration *(13)* is carried out as follows:

One-half gram samples are wetted with 2 ml. alcohol and dispersed by adding 100 ml. of water. This sol is titrated to a faint pink of phenolphthalein with NaOH, then 5.0 ml. of neutral 40 percent formaldehyde are added and after 2 or 3 minutes, a further titration to neutrality is made. The first titration represents free carboxyls and the second shows the ammonium salts. The solution may then be given the saponification titration for methoxyl determination, as mentioned above.

Combining Weight. The combining or equivalent weight of practical significance, that is the weight in grams which can react with one equivalent or 20 grams of calcium, is determined from the titration of a sample which has been washed in acid-alcohol as described by Olsen and associates *(11)*. The acid- alcohol treatment frees the carboxyls of ammonium and any metallic ions so that the resultant titration actually reveals the true combining capacity. The remaining carboxyl groups are either esterified or combined as acid amides.

The titration of ammonia resulting from the distillation of the pectinate in a sodium hydroxide solution gives a value that represents both the content of ammonium salts and acid amide groups. This titration value added to the initial, saponification and ash titrations leads to a combining weight representing the pectic nucleus.

Percent Esterification. The theoretical percent esterification has been calculated as shown in Table 1. It is possible, in the case of pectinates like those here discussed (or only after acid-alcohol rinsing for pectinates with high ash or considerable "ballast"), to divide the determined percent methoxyl by 0.1632 and get the percent esterification. The value so obtained will be approximately the same as the one derived by determining the ratio of the saponification titer to the total titer (initial, NaOH distillation, methoxyl or saponification and ash). The fact that these values all agree so closely adds evidence that the assumptions of Table 1 are not far from correct.

Hills and Speiser *(6)* and Speiser *(16)* have given rather complicated procedures for calculating the percent esterification. The procedure suggested by McDowell *(9)* is cumbersome and designed particularly for use on pectin extracts. It is obvious from what has been said and from the data and curves which follow, that these methods, particularly those of Speiser *(16)* and of Hills and Speiser *(6)*, do not apply to a type of pectinate such as is here described.

Data Illustrating Points of Structural Differences Between Various Types of Low Methoxyl Pectinates

The curves and points on Figure 2 are convincing evidence that the ammonia-alcohol demethylated pectinates are quite different, structurally, from pectinates heretofore described in the literature, when compared as to methoxyl and combining weight. The combining weights plotted in Figure 2 were calculated from the initial titration on acid-alcohol rinsed samples. Although not shown in Figure 2, the sodium hydroxide demethylated pectinates gave values practically coinciding with those shown for the acid demethylated samples of Kaufman, Fehlberg and Olsen *(7)*. The points representing ammonia-alcohol demethylated samples were chosen from samples made by widely differing ammonia concentrations and different treating times, to illustrate how the relations between methoxyl and combining weight may be shifted almost at will. The ammonia-pectin concentrations in these cases were far higher than were used by Schultz, Lotzkar, Owens and Maclay *(15)* for their samples illustrated in Figure 2.

Data on six ammonia-demethylated pectinates are presented in Table 2 to illustrate further some of the points already discussed.

The values for percent esterification, calculated on the original samples, by the method of Hills and Speiser *(6)*, are exceedingly high, and are represented by the six circles at the right side of Figure 3. When those same calculations are made on acid-alcohol rinsed portions of four of the same samples, the values are lowered considerably (due to ammonium ions on carboxyls being taken into account) as shown by the center set of points (A, B, C and D) in Figure 3. The values do not yet take into account the acid amide groups such as is done when the sodium hydroxide distillation titer is included and as is shown by the group of points near the curve on Figure 3. These latter points represent the values on the six "Untreated Samples" of Table 2, calculated from $-OCH_3$ titer/total titer.

It is interesting to notice in Table 2 that values for the percent esterification calculated from $\% - OCH_3$/

TABLE 2

Combining Weight and Percent Esterification Data for Six Ammonia-Alcohol Demethylated Pectinates

Sample Number	Untreated Samples [1]							Acid-Alcohol Rinsed Samples [1]			
		Percent Esterification			Conc. NH_3 in Mfg.[k]	Combining Weight		Combining Weight		Percent Esterification	
	% $-OCH_3$	Hills-Speiser Method	$-OCH_3$ Titer / Total Titer [l]	% $-OCH_3$ / 0.1632		Free Carboxyls and Ammonium Salts	If Amide Groups Included	Olsen et al. Method	If Amide Groups Included	Hills-Speiser Method	$-OCH_3$ Titer / Total Titer [l]
A	4.2	59.9	28.2	25.7	1	417	289	409	288	36.2	28.6
B	4.6	67.1	30.8	28.2	2	488	303	482	305	40.7	30.2
C	4.3	67.3	29.7	26.4	4	505	302	520	309	42.7	30.6
D	4.4	69.3	30.3	27.0	8	552	311	560	309	44.2	30.5
E	4.0	65.6	25.9	24.5	8	529	273	.....	.....	.....	.....
F	3.0	56.4	19.5	18.4	8	508	249	.....	.....	.....	.....

[1] Data are on ash-moisture-free basis.
[j] Data on moisture-free basis.
[k] Given in relation to amount used for first sample, other ingredients kept constant, time only varying.
[l] Total titer includes: initial, saponification, sodium hydroxide distillation, and ash titers.

0.1632 are reasonably close to those calculated by using the $-OCH_3$ titer and the total titer. Surely these pectic molecules can not be greatly different from the one assumed for Table 1.

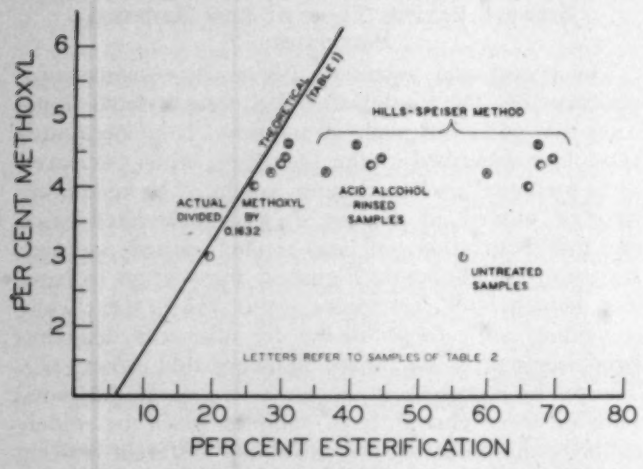

FIG. 3. Relation of percent methoxyl and percent esterification for ammonia demethylated pectinates, as determined by different methods.

These same points are again illustrated by considering combining weights and methoxyl as shown in Figure 4, for the six pectinates of Table 2. The combining weights calculated in the usual way from data on acid-alcohol rinsed samples agree, within the experimental error, with the values from the untreated samples and obtained by combining the initial titer with the one following addition of neutral formaldehyde.

When the initial titer is combined with the one for the sodium hydroxide distillation (to take into account the acid amide) the resultant "combining weights" are quite close to those given by Olsen and his associates *(11)* as the theoretical (see the points near the curve on Figure 4).

The relative numbers of the various groups which occur on the different types of pectinate may be estimated from titration data, as illustrated by the following figures for the ammonia-demethylated pectin "B" of Table 2. Titration values are as follows, for that pectinate, calculated as ml. 0.10 N reagent per gram of moisture-free sample:

A. Initial, to faint pink with phenolphthalein	7.3 ml.
B. After adding neutral 40% formaldehyde	13.3 ml.
C. Saponification titer (methoxyl determination)	14.7 ml.
D. Distillate titer after distillation from NaOH solution	25.8 ml.
E. Ash titer	0.6 ml.
F. Amide "Titer" (D-B)	12.5 ml.
Total titer (A + C + D + E)	48.4 ml.

When titration values A, B, C, E and F are calculated as percentages of the total titer, the percentages are indicative of the distribution of the groups represented in one hundred galacturonide units. The percentages thus calculated for "B" of Table 2 are:

Free carboxyl groups	15.1%
Ammonium salts on carboxyls	27.6%
Methoxyl groups	30.4%
Acid-amide groups	25.8%
Carboxyls as metal salts	1.2%
Total carboxyls indicated	100.0%

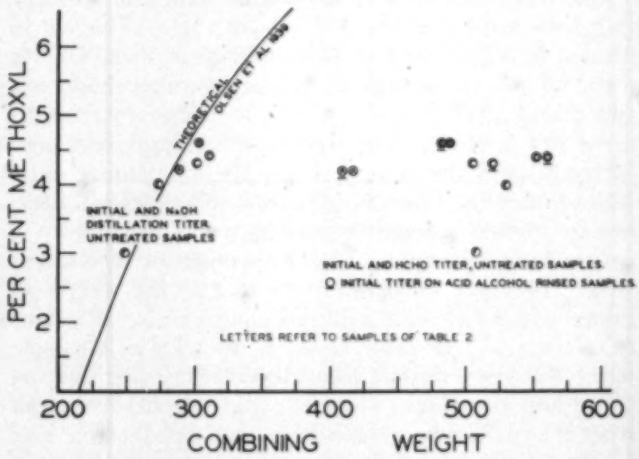
PER CENT METHOXYL
COMBINING WEIGHT
THEORETICAL OLSEN ET AL. DATA
INITIAL AND NaOH DISTILLATION TITER, UNTREATED SAMPLES
INITIAL AND HCHO TITER, UNTREATED SAMPLES
INITIAL TITER ON ACID ALCOHOL RINSED SAMPLES
LETTERS REFER TO SAMPLES OF TABLE 2

FIG. 4. The combining weight of ammonia demethylated pectinates, with and without consideration of amide group.

Similar values, calculated in this manner and rounded to even figures, for four types of pectinates are shown in Table 3. It must be kept in mind that the values in Table 3 are for single samples of the various types of pectinates. Similar data for check samples may vary by several units.

The products made by enzymes, acid or fixed alkalis usually show a large proportion of carboxyl groups involved as metal salts. Sample D-46, even though

TABLE 3

Relative Number[m] of Various Groups in Typical Pectinates. Based Upon 100 Galacturonide Units

Structural Group	Pectin	Low Methoxyl Pectinates Made by Different Methods				
		Enzyme (Citrus) D-89	Acid (Citrus) D-46	Acid (Apple) G.F. 320	NaOH (Citrus) C-2756	Ammonia (Citrus) "B" Table 2
— COOH Free Carboxyl	15	8	28	31	9	15
— $COONH_4$ Ammonium Salt	17	1	47	....	9	28
— $COOCH_3$ Methyl Ester	65	23	22	29	31	30
— COOX Metal Salt	3	68	2	40	51	1
— $CONH_2$ Acid Amide	....	....	1	....	....	26

[m] Calculated from titration data. This table has been presented merely to illustrate, in a rough manner, the general proportion of the indicated groups. Small variations in preparative technic for pectinates may alter these values by several units.

made by high acid hydrolysis, was prepared from a low-ash pectin such as is shown in Table 3, and consequently has a low ash titer but an unusually large content of carboxyl groups combined with ammonium ions due to neutralization with large amounts of ammonium hydroxide. The very small content of acid amide is no doubt due to this use of large volumes of ammonia.

The hypothesis that acid amide groups are present in pectinates such as here described, is substantiated when one reviews the following facts. There is always more nitrogen revealed by distillation after sodium hydroxide addition than is indicated by the titration after adding neutral formaldehyde. The amount of ammonium salt indicated by the titration after adding formaldehyde checks the increase in initial titer after the acid-alcohol rinse of these samples. A Van Slyke titration on a sample recovered from a calcium gel and saponified with excess fixed alkali, checks the titer obtained by subtracting the titer after formaldehyde from that of the sodium hydroxide distillation process. When the environment during demethylation is most conducive to the presence of increased amounts of NH_3 rather than of the $—NH_4$ ion, such as in alcoholic systems, the content of amide groups increases, in keeping with the fact that acid amides are considered to form when ammonia reacts with esters. The milligrams of calcium ion per gram of pectinate required for optimum gel formation remain practically constant even though the ammonia concentration during demethylation was increased 8 fold with a resultant increase in acid amide groups. Samples of these pectinates have been recovered from calcium gels and have been examined by the various technics mentioned above. The data indicate that the calcium ions react with free carboxyls and some of the ammonia neutralized carboxyls. The amide nitrogen content, before and after calcium gelation, remains essentially constant.

Practical Use of Calcium Salts for Gel Formation

The four types of low methoxyl pectin described above make suitable gels (when the calcium content is optimum) when present at 0.5-1.0 percent concentration. Variations in gel-forming ability occurring as the result of fluctuations in fruit sources, or in manufacturing processes, can be taken care of by diluting the pectin with sufficient dextrose so that 1.0 percent of the standardized material is always required for a standard gel. The calcium needed in water gels of this type, expressed as milligrams of calcium ion per gram of the pectin, is usually 4-10 for enzyme type pectins, 15-30 for pectins made by the ammonia-alcohol process (Exchange Pectin L. M.), and from 30-60 for the ones made by the high acid method.

Commercial usage of low methoxyl pectin, Pectin L. M., demands that the product be uniform in its gel forming ability and in its calcium requirement. Therefore, factory standardization is based upon the performance of the Pectin L. M. in a special test gel with a fixed level of calcium ions. The test gel batch is designed to produce 600 grams of gel containing 30 percent added sugar, 1.0 percent Pectin L. M., 0.1 percent sodium citrate dihydrate, 25 milligrams of calcium per gram of Pectin L. M. and acid to produce a final pH of 3.0 ± 0.05. Such a gel is considered standard when the Exchange Ridgelimeter *(3)* shows a sag between 20 and 21 percent. Sodium citrate is used in these test gels but is usually not necessary in most gels containing fruit or berry juices, or with milk.

Since calcium is responsible for gelation it is evident that one would expect to obtain the best solution of the pectin when no calcium was present. This means that the ideal way to get a gel would be to dissolve the pectin first, then, while the sol is hot, add a solution of some calcium salt. When such a sol cools to room temperature a gel of good texture results. This procedure is not always desirable from a practical viewpoint since it may sometimes be necessary to have the calcium salt present with the pectin at the start of the operation. It is for this reason that slowly or slightly soluble calcium salts are desirable, such as the phosphates. Monocalcium phosphate, $Ca(H_2PO_4)_2 \cdot H_2O$, and tricalcium phosphate are good salts to use, because with these there is not enough calcium immediately available to interfere with solution of the pectin.

When using low methoxyl pectin such as Exchange Pectin L. M., the amounts of these two calcium salts needed vary from 3 to 15 percent for the monocalcium phosphate and from 1 to 7 percent for the tricalcium phosphate, based upon the weight of Pectin L. M. when the latter is used at 1.0 percent of the gel weight. When milk is used instead of water in forming gels with Pectin L. M. no added calcium is needed. It is merely necessary to add the Pectin L. M. to the milk at 1.0 percent concentration, heat to 60-70° C., then cool to get the gel. When possible it is best to dissolve the Pectin L. M. in a small amount of hot water then to add cold milk. This permits good solution of the Pectin L. M. and minimizes heating of the milk.

Many fruit products contain sufficient natural calcium salts to provide the Pectin L. M. with part of its requirement for gelation. It is usually well to assume, in working out a new recipe using fruit, that nearly half the calcium will be supplied by the fruit and to add enough of an appropriate calcium salt to furnish 12-18 milligrams of calcium per gram of Pectin L. M. (this corresponds to about 8-12 percent as much monocalcium phosphate as is used of Pectin L. M.).

It is desirable to think of the calcium requirement in terms of the ratio of calcium/pectin in milligrams per gram . Pure water gels require this value to be 25 while with fruits, where calcium is already present, this value will be between zero and 25.

Summary

Pectinates containing acid amide groups are made by the partial demethylation of pectin with ammonia in alcoholic systems or in concentrated aqueous ammonia. The three previously recognized de-esterifying agents for pectin are: Acids; fixed alkalis or dilute aqueous ammonia systems; and enzymes, alone or in connection with alkalis. Pectinates made by these last three methods have essentially the same structure but exhibit characteristic differences in such properties as viscosity, calcium sensitivity and gel power.

A proximate analysis of the pectinate described in the present report shows that for each free carboxyl there are two combined as the ammonium salt; two as the methyl ester; and two as the acid amide. This type of pectinate, although available commercially for a number of years, has not been differentiated, in the literature, from the type made by ordinary alkaline de-esterification. The influences of the ammonium ion and the acid amide group upon the usual laboratory determinations of degree of esterification and of combining weight are discussed.

LITERATURE CITED

1. Bonner, J. Chemistry and physiology of the pectins. *Botan. Rev.*, **2**, 475 (1936).
2. Carson, J. F. Some polygalacturonide n-alkylamides. *J. Am. Chem. Soc.*, **68**, 2723 (1946).
3. Cox, R. E., and Higby, R. H. A better way to determine the jellying power of pectins. *Food Ind.*, **16**, 441 (1944).
4. Deuel, H., and Neukom, H. Derivatives of pectic acid as models of proteins. *Nature*, **159**, 882 (1947).
5. Fellenberg, Th. von. The pectins. *Mitt. Lebens. Hyg.*, **5**, 225 (1914).
6. Hills, C. H., and Speiser, R. Characterization of pectin. *Science*, **103**, 166 (1946).
7. Kaufman, C. W., Fehlberg, E. R., and Olsen, A. G. Chemists adapt pectins to new industrial uses. *Food Ind.*, **14**, 57 (1942).
8. Kertesz, Z. I., Baker, G. L., Joseph, G. H., Mottern, H. H., and Olsen, A. G. Report of the committee for the revision of the nomenclature of pectic substances. *Chem. Eng. News*, **22**, 105 (1944).
9. McDowell, R. H. (to H. P. Bulmer & Co., Ltd.) British Patent 541,528. (Dec. 1, 1941).
10. National Formulary, 8th Ed., 1946. American Pharmaceutical Association, Washington, D. C. 850 pp.
11. Olsen, A. G., Stuewer, R. F., Fehlberg, E. R., and Beach, N. M. Pectin studies: Relation of combining weight to other properties of commercial pectins. *Ind. Eng. Chem.*, **31**, 1015 (1939).
12. Romeo, Giovanni. Determination of the methoxyl groups in pectin. *Ann. Chim. Applicata*, **23**, 530 (1933).
13. Ronchése, A. A new method for the determination of ammonia. *J. Pharm. Chim.* (6) **25**, 611 (1907).
14. Schneider, G. G., and Fritschi, U. On the esterification of pectin materials. IV. The constitution of pectic-ester. *Ber.*, **70**, 1611 (1937).
15. Schultz, T. H., Lotzkar, H., Owens, H. S., and Maclay, W. D. Influence of method of deesterification on the viscosity and acid behavior of pectinic acid solutions. *J. Phys. Chem.*, **49**, 554 (1945).
16. Speiser, R. Advances in pectin chemistry. Part I. *J. Polymer Sci.*, **2**, 281 (1947).
17. Stuewer, R. F., and Olsen, A. G. Pectin studies. V. Organic base derivatives of pectinic and pectic acids. *J. Am. Pharm. Assoc.*, **29**, 303 (1940).

Enzyme-Demethylated Pectinates and Their Gelation[a]

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Explanations are offered for the difference in the type of gelation occurring in pectinic acids which have been de-esterified, respectively, by enzymes and by acids.

Several years ago this laboratory undertook a study of methods for preparing low-ester pectin from apple pomace as a possible means of increasing the utilization of apple processing wastes. The preparation of low-ester pectins by acid-deesterification had been previously studied by Olsen, et al. *(8)* and by Baker and Goodwin *(1)*. The acid method appeared to possess several disadvantages for commercial usage; it is time consuming, requires acid resistant equipment, and causes some degradation of the product.

The enzyme pectase was considered as a possible catalyst for the deesterification of pectin because the milder conditions of acidity and more rapid rate of reaction would be advantageous in a commercial process.

The procedure as developed at our laboratory has been described in previous publications *(5, 7)* and is briefly as follows: A concentrated pectin extract prepared from dried apple pomace is treated with tomato pectase at pH 6.5 and 30° to 40° C. for an hour or less, depending on the concentration of enzyme and the extent of de-esterification desired. The reaction is stopped at the desired point by acidifying the mixture and heating to destroy the enzyme. The product may be isolated by precipitation with ethanol or iso-propanol, and dried in the usual manner.

The pectin complex consists of long straight chain molecules of partially methyl-esterified polygalacturonic acid (Fig. 1) to which are attached at undetermined points side groups of araban and galactan. The molecular weight ranges from relatively low values to approximately 300,000. The araban and galactan contents of a sample may vary from 0 to 30 percent and 0 to 40 percent, respectively, depending upon the source, method of extraction, and subsequent treatment. The methyl ester content (calculated as CH_3O) may vary from approximately 7 to 12 percent for non-deesterified pectins to 0 for pectic acid.

Because of the variable amount of non-galacturonide

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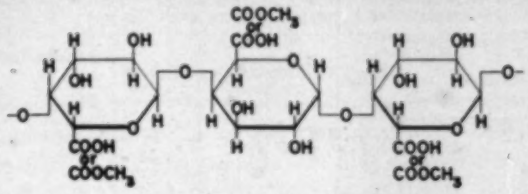
CONSTITUTION AND STRUCTURE OF PECTINIC ACIDS



POLYGALACTURONIDE CHAIN

Degree of esterification	0 to 100%
Araban content	0 to 30%
Galactan content	0 to 40%
Average Molecular Weight	Up to 300,000

FIG. 1. Constitution and structure of pectinic acids. From Speiser, Hills, and Eddy *(15)*.

material present in various preparations of apple pectin, it is more accurate to characterize a preparation by expressing the percent esterification of the polygalacturonide chain than by the weight percent of methoxyl *(4)*. Non-deesterified apple pectin is approximately 70 percent methyl esterified and will form a gel only in the presence of a suitable concentration of sugar and of acid. Pectins of less than 50 percent esterification form the conventional sugar-acid gels, but in addition will gel in the presence of a suitable concentration of a polyvalent cation, such as calcium, in the absence or presence of sugar.

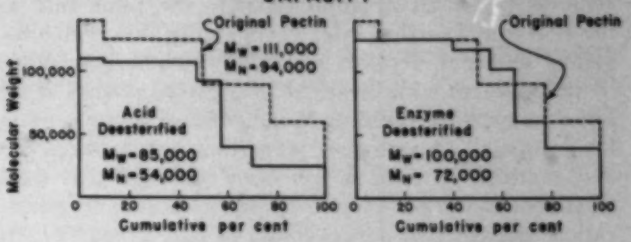
Thus it is possible to distinguish two different types of pectin gels; (1) hydrogen-bonded gels, such as the usual pectin-sugar-acid jellies; and (2) ionic-bonded gels made with a polyvalent cation such as calcium. The strength of hydrogen-bonded pectin gels depends principally on the molecular weight. The strength of calcium-pectinate gels is influenced also by the mode of deesterification. In general, enzyme-deesterified pectinic acids make weaker gels than do acid-deesterified *(5, 13)*. The present paper will describe some of the properties of enzyme-deesterified pectinic acids and attempt to explain the reasons for their inferior gel strength. Various aspects of this study have been reported in detail in previous publications from this laboratory *(4, 13, 14)*.

Molecular Weight

It is difficult to determine the molecular weight of pectin by viscosity in aqueous solution because of the complex behavior of charged particles in a water solution. To avoid these complications, Schneider and co-workers *(2, 10, 11)* nitrated pectin and measured molecular weights by viscosity in acetone solution, a procedure analogous to that commonly used in cellulose chemistry. It is further possible to fractionate the nitrated pectin and to determine the viscosity and molecular weight of each fraction and to calculate the weight-average and number-average molecular weights.

In Fig. 2 are presented histograms showing the molecular weight distribution of a high-ester pectin and two deesterified pectins derived from it by acid and enzyme deesterification, respectively. The total area under each step diagram is equal to the weight average molecular weight. The homogeneity of a sample can be estimated by comparing the weight average molecular weight with the calculated number average molecular weight. It can

MOLECULAR WEIGHT DISTRIBUTION & GELLING CAPACITY



Method of Preparation	Degree of Esterification	Methoxyl (CH_3O)	Gel Strength 65% Sugar Jellies	Gel Strength Calcium Pectinate Gels
Acid Deesterified	32%	4.71	20cm.	30cm.
Enzyme Deesterified	36	4.80	47	13
Original Pectin	80	8.87	61	No Gel

FIG. 2. Molecular weight distribution and gelling capacity. From Speiser and Eddy *(14)*.

be seen that the enzyme-deesterified product has undergone slight degradation whereas the acid-deesterified pectinic acid has been degraded to a considerably greater extent. It is also apparent that the strength of the 65 percent sugar jellies prepared from these three pectins is in the same order as their molecular weights. Although the enzyme-deesterified pectinic acid produced a stronger hydrogen-bonded gel than the acid-deesterified sample, it produced a much weaker calcium-pectinate gel. Therefore, the molecular weight is not a sufficient index of the gelling power when comparing acid and enzyme-deesterified pectins.

Non-Galacturonide Materials

It has been shown by Schneider and Bock *(9)* that the characteristic properties of pectin—gelation, film formation, and high viscosity in dilute aqueous solution—derive from the polygalacturonide chain; the non-galacturonide constituents or "ballast materials," chiefly araban and galactan, act mainly as diluents.

Enzyme deesterification causes the removal of very

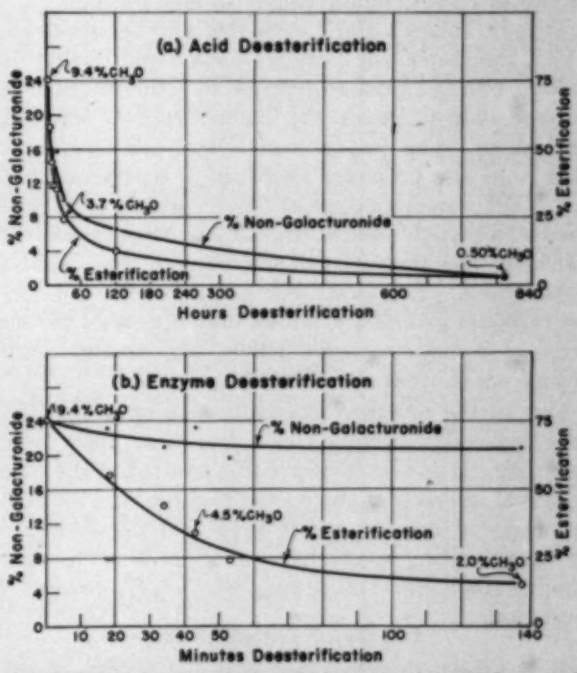


FIG. 3. Rate of removal of methyl ester and non-galacturonide materials during deesterification. From Hills and Speiser *(4)*.

little ballast whereas acid deesterification leads to the removal of ballast at approximately the same rate as the removal of methyl ester groups (Fig. 3). Since the gelling tendency depends principally on the percentage of polygalacturonide material in a given sample, if all other factors are equal, acid-deesterified pectins, gram for gram, will give stronger jellies than enzyme-deesterified pectins. In fact, it has been observed that mild treatment of pectin followed by precipitation and purification results in an improvement of jelly grade *(1)*. It is also possible that during precipitation and purification of acid-treated pectins, low molecular weight fractions would be washed out. This would also tend to increase the grade of the product, but at the expense of reduced yields.

Solubility

Acid- and enzyme-deesterified pectinic acids show marked differences in solubility. Pectic acids or pectinic acids of low degree of esterification prepared by acid deesterification are relatively insoluble in water. Usually a small proportion of the sample will go into solution but the addition of more solvent will not cause additional material to dissolve. This behavior is characteristic of crystalline high polymers such as cellulose, where the alignment of long rigid chains permits the interaction of hydrogen bonding groups to form strongly knit aggregates which require considerable energy to disrupt.

Pectic substances with low degree of esterification and consequently a high concentration of strongly interacting carboxyl groups tend to crystallize upon precipitation or drying and form relatively insoluble aggregates. If there are bulky side groups, or if irregularities of a sufficiently high degree are present in the polymer chains, a lesser extent of crystallization results, and aggregates are formed which admit solvent more freely and dissolve more readily. Enzyme-deesterified pectinic acids, because of their high non-galacturonide content are much more soluble in water than acid-deesterified pectins of corresponding degree of esterification.

Data in Tables 1 and 2 show that acid deesterification, which simultaneously removes ballast and methyl ester groups, effects a greater decrease in solubility than does enzyme, which leaves the ballast content essentially unchanged. The presence of a few percent of alkali metal ions also promotes solubility. Preparations H88F and H89F, containing about 5 percent ash, were readily soluble although almost completely deesterified. De-ashing to less than 0.5 percent reduced their solubility considerably. The electrolyte promotes dissociation of the carboxyl groups and so not only decreases the number of strong hydrogen bonds but creates ionized groups which repel one another.

One of the practical factors discouraging the use of very low ester pectinic acids has been their limited solubility. It is now obvious that control of the degree of lateral interaction among the pectinic acid molecules, by choice of enzyme rather than acid deesterification, by incorporating electrolyte or other diluent, and by rapid precipitation and drying, can largely remove this limitation.

Degree of Esterification

It has been demonstrated that the ability of dilute pectin solutions to form calcium-bonded gels, both in the presence and in the absence of sugar, depends on the degree of esterification *(3, 4, 5)*. In 1 percent solutions pectins with greater than 50 percent esterification do not gel with calcium ion alone. The lower the degree of esterification the smaller is the amount of calcium required to form the gel of optimum strength, because of the greater opportunity for forming cross links. The relationship between degree of esterification and calcium requirement for gel formation is shown in Table 3. In this table the percent methoxyl has been used instead of percent esterification since sufficient data were lacking for computation of the latter values.

It is interesting to note that the enzyme-deesterified pectins increased in gel strength, whereas the acid-deesterified pectins decreased in gel strength, as the methoxyl values decreased. The decrease in gel strength of the acid-deesterified series would be expected since acid causes degradation of the polygalaturonide chain. The increase in gel strength of the enzyme-deesterified

TABLE 1

Solubility of Acid-deesterified Pectinic Acids. Two-gram samples shaken with 100 g. water for 2 hrs. at 27° C. and 100° C.

Sample	Ash	Degree of esterification	Ballast content	Amount dissolved	
				27° C.	100° C.
	%	%	%	%	%
H91	0.25	75	22.2	100	100
H91A	0.22	57	15.0	100	100
H91C	0.20	35	14.5	100	100
H91D	0.40	24	8.2	31	100
H91E	0.53	11	4.7	20	50
H91K	0.37	3	0.9	5	39

Data from Speiser, Copley, and Nutting *(13)*.

TABLE 2

Solubility of Enzyme-deesterified Pectinic Acids. Two-gram samples shaken with 100 g. water for 2 hrs. at 27° C.

Sample	Ash	Degree of esterification	Ballast content	Amount dissolved at 27° C.	Amount of de-ashed material dissolved at 27° C.
	%	%	%	%	%
H91F	0.31	55	18.3	100	
H91G	0.51	45	17.4	100	
H91H	0.55	33	19.7	100	
H91I	0.62	23	16.4	100	100
H91J	1.01	14	16.2	100 (cloudy)	85 (cloudy)
H88F	4.69	3	21.5	100	70 (about)
H89F	4.95	4	19.1	100	74

*De-ashed to 0.5 percent.
Data from Speiser, Copley, and Nutting *(13)*.

TABLE 3

Effect of methyl ester content of pectinate on optimum Calcium-pectinate ratio and on gel strengths

CH_3O	Method of deesterification	Gel strength cm. H_2O	Calcium pectinate ratio at optimum
%			
8	Enzyme	16	0.040
7	Enzyme	26	0.020
6	Enzyme	33	0.019
5	Enzyme	37	0.017
7.7	Acid	8	0.073
6.1	Acid	270	0.070
4.9	Acid	180	0.038
4.4	Acid	110	0.029
3.8	Acid	84	0.023

Data from Hills, White, and Baker *(5)*.

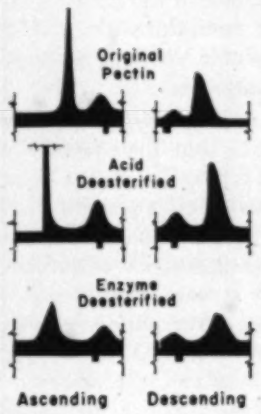
series is due to the increase in homogeneity with respect to charge distribution of the molecules within a given sample on continued deesterification.

Charge Distribution

One must remember that the degree of esterification of a pectin sample is an average quantity. Individual molecules in a given preparation may show values considerably higher or lower than the average. Acid and alkali presumably remove ester groups at random, so that each individual molecule has about the same degree of esterification as the average for the entire sample. From our knowledge of the action of enzymes, we would not expect pectase to act in a random manner. To confirm this point, electrophoretic studies were made of a sample of high-ester pectin and of acid- and enzyme-deesterified pectins prepared from it. Since the electrophoretic mobility of a pectin molecule is proportional to charge density, any heterogeneity in degree of esterification should be revealed in the electrophoresis patterns.

Figure 4 shows typical patterns for acid- and enzyme-deesterified pectins and for the pectin from which they

ELECTROPHORESIS PATTERNS FOR ACID AND ENZYME DEESTERIFIED PECTINIC ACIDS



	Degree of Esterification	Gel Strength 65% Sugar Jellies	Gel Strength Calcium Gels
Original Pectin	80%	61 cm.	No Gel
Acid Deesterified	32	20	30 cm.
Enzyme Deesterified	36	47	13

FIG. 4. Electrophoresis patterns for acid and enzyme deesterified pectinic acids. From Hills, Mottern, Nutting, and Speiser *(3)*.

were prepared. Both non-deesterified and the acid-deesterified pectin gave sharp boundaries indicating homogeneity of the molecules within each preparation. The broad pattern for the enzyme-deesterified pectin indicates a marked heterogeneity of the molecules with respect to degree of esterification. Ward, Swenson and Owens *(16)* have made similar observations on citrus pectin. They fractionated enzyme-deesterified pectins by chemical means and found marked differences in the degree of esterification of the fractions.

Previously it was pointed out that the amount of calcium ion required for maximum gel strength varied with the degree of esterification. Since most of the molecules in a sample of acid-deesterified pectinic acid would have about the same degree of esterification, a single calcium concentration would be optimum for all the individual molecules and the maximum gel strength would be achieved. However, a single calcium concentration could not be optimum for all the molecules of an enzyme-deesterified pectinic acid. The fraction with a low degree of esterification would require less calcium than the fraction with a higher degree of esterification. An intermediate calcium concentration would precipitate the former while incompletely cross-linking the latter. It is principally for this reason that enzyme deesterified pectins are inferior in their gel making properties to deesterified pectins produced by acid catalysis.

This explanation was confirmed by the following experiment: Two pectinic acid preparations produced by acid-deesterification, with methoxyl contents of 3.3 and 5.7 percent, gave calcium gels with strength of 88 and 89 cm. The pectinic acids were mixed in equal amounts, giving a methoxy content of 4.5 percent. The calcium gel strength was only 44, or half that of the gel made from each constituent of the mixture. The distribution in degree of esterification was no longer sharp but resembled that of an enzyme-deesterified pectin. A similar mixture made with enzyme-deesterified pectinic acids did not give a correspondingly weakened gel.

This explains the increase in gel strength of the enzyme products with progressive deesterification as observed in Table 3. As the average degree of esterification becomes smaller, the heterogeneity of the molecules inevitably becomes less and the gel strength rises until syneresis reverses the trend.

The possibility should be mentioned that the enzyme may cause another type of non-random demethylation, in which one end of the molecule would be completely deesterified, resembling pectic acid, and the rest of the molecule would be unaltered. Such a condition would also contribute to the observed difference in electrophoretic mobility and gel behavior of the acid and enzyme products. Jansen and MacDonnell *(6)* have accumulated experimental evidence that such hybrid pectic acid-pectin molecules actually result from pectase action, and separate evidence from data by Schultz et al. *(12)* tends to confirm this conclusion.

Summary

The difference in gelation characteristics of enzyme- and acid-deesterified pectinic acids can be explained on the basis of fundamental differences in composition and structure of the two products.

Acid catalysis causes the simultaneous removal of methyl ester groups and non-galacturonide materials. Enzyme deesterification does not affect the non-galacturonide materials, and for that reason the enzyme-prepared pectinic acids are more readily soluble in aqueous solution.

Enzyme-deesterified pectinic acids show broad electrophoretic patterns indicating a marked heterogeneity of the molecules within a given preparation with respect to degree of esterification. Non-deesterified and acid-deesterified pectinic acids are more nearly homogeneous in this respect. This accounts for the inferior gel strength of the enzyme-deesterified product. With increased deesterification, the enzyme product increases in gel strength because of increased homogeneity, but the acid-deesterified product decreases in gel strength because of reduction in molecular weight.

REFERENCES

1. BAKER, G. L., AND GOODWIN, M. W. Delaware Agr. Expt. Sta. *Bull.* 234 (1941).

2. Henglein, F. A., and Schneider, G. *Ber.* **69***B*, 309 (1936).
3. Hills, C. H., Mottern, H. H., Nutting, G. C., and Speiser, R. 108th Meeting of the American Chemical Society, New York, Sept., 1944.
4. Hills, C. H., and Speiser, R. *Science*, **103**, 166 (1946).
5. Hills, C. H., White, J. W., and Baker, G. L. *Proc. Inst. Food Tech.*, 1942, p. 47.
6. Jansen, E. F., and MacDonnell, L. R. *Arch. Biochem.*, **8**, 97 (1945).
7. Mottern, H. H., and Hills, C. H. *Ind. Eng. Chem.*, **38**, 1153 (1946).
8. Olsen, A. G., Stuewer, R. F., Fehlberg, E. R., and Beach, N. M. *Ind. Eng. Chem.*, **31**, 1015 (1939).
9. Schneider, G., and Bock, H. *Ber.* **70***B*, 1617 (1937).
10. Schneider, G., and Fritschi, U. *Ber.* **69***B*, 2537 (1936).
11. Schneider, G., and Ziervogel, M. *Ber.* **69***B*, 2530 (1936).
12. Schultz, T. H., Lotzkar, H., Owens, H. S., and Maclay, W. D. *J. Phys. Chem.*, **49**, 554 (1945).
13. Speiser, R., Copley, M. J., and Nutting, G. C. *J. Phys. and Colloid Chem.*, **51**, 117 (1947).
14. Speiser, R., and Eddy, C. R. *J. A. C. S.*, **68**, 287 (1946).
15. Speiser, R., Hills, C. H., and Eddy, C. R. *J. Phys. Chem.*, **49**, 328 (1945).
16. Ward, W. H., Swenson, H. A., and Owens, H. S. *J. Phys. Colloid Chem.*, **51**, 1137 (1947).

Recent Developments of Practical Significance in the Field of Pectic Enzymes[a]

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It would appear that changes leading to loss of pectinic substances in processed tomato products are the result of the action of tomato pectin-methylesterase (PM) and a pectic acid depolymerase (DP). The pectinic acids are first demethylated by the PM, and the DP then rapidly depolymerizes the pectic acid substrate made available to units approaching pentagalacturonic acid. The colloidal properties of the tomato product are thus lost and poor consistency results.

It has been demonstrated that consistency is one of the important factors determining the organoleptic qualities of some tomato products such as tomato paste, purée and tomato juice *(1)*. Previous work in this laboratory has shown that a desirable consistency along with freedom from "settling" is closely related to the pectinic acid content of the finished product. In turn the pectinic acid content is largely determined by the action of the pectic enzymes of the tomato during the process of manufacture.

Two general commercial processes are employed in the manufacture of tomato juice. In one process ("hot break") the tomatoes are macerated at a temperature of 180° F. (82° C.) or better; while in the other ("cold break"), the tomatoes are macerated at some temperature below 180° F. (82° C.) The "hot break" process produces a juice with high pectinic acid content having a pleasing consistency and not inclined to "settle." The "cold break" process results in a thin watery juice having an almost negligible pectinic acid content and very inclined to settle on standing.

Wildman *(13)* and Smith *(11)* noted this loss in the pectinic acid content of "cold break" tomato juices and attributed it to the action of pectic enzymes present which were inactivated by the "hot break" process. They also noticed that this loss was extremely rapid, occurring in the first few minutes after the tissues were macerated.

With the discovery about ten years ago *(2)* that tomatoes contained only a little pectin-polygalacturonase (PG) but were a rich source of pectin-methylesterase (PM), an attempt was made to explain the rapid loss of pectic substances in macerated tomato tissues on the basis of the action of the latter enzyme *(1)*. It was postulated *(1)* that upon maceration of the tomatoes the PM rapidly demethylated the pectinic acids yielding pectic acid which formed insoluble calcium salts and was then removed from the serum.

Although studies of the PM of the tomato and other higher plants *(4, 6)* have shown that they possess an optimum pH for activity at pH 6.5 to 7.5, it was shown by Lineweaver et al *(4)* that at least 50 percent of the maximum activity at the optimum pH could be obtained at pH values as low as 4.5 in the presence of sufficient amounts of cationic salts. The tomato PM is present in the intact fruit in large quantities and is strongly bound in the tissues showing no activity in situ. Upon maceration of tomato tissue, however, the enzyme is released and the salts content of the tomato is sufficiently high to render the PM quite active. It is thus capable of rapidly demethylating the pectic substances present producing pectic and pectinic acids. The "hot break" process inactivates the PM and prevents demethylation of the substrate.

Further work has shown, however, that the demethylated pectic substances are not then lost by precipitation as insoluble calcium salts as was originally supposed. Numerous analyses have been carried out on the 70 percent ethanol insoluble solids of both "hot" and "cold break" juices with the result that the supposed calcium pectates were not found. Ammonium oxalate extraction followed by calcium pectate determinations never recovered more than 10 percent of the original pectic substances which had been lost. On the other hand analyses of the same samples by the CO_2 method of Whistler, Martin and Harris *(12)* gave a recovery of 80 to 90 percent of the original pectic substances. It thus appeared that the pectic substances had been altered by transformations other than demethylation.

Accordingly the PG reported in tomatoes by various authors *(5, 7, 9)* was reinvestigated to establish its role on the loss of pectic substances. The original findings of

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Kertesz *(2)*, that the PG was present in tomatoes in only small proportions were readily established. On the basis of the rate of increase in reducing power when this enzyme acts on a pectic acid substrate at pH 4.5 (optimum pH of the tomato PG), the PG content of 100 ml. of the tomato macerate corresponds to the PG activity of only 70 mg. of a commercial pectinase preparation (Pectinol 100-D) obtained from mold organisms. This amount of PG activity, if exerted in the same manner as that of commercial pectinases, is not sufficient to account for the very rapid loss of the pectic substances of the tomato which is known to occur in the first few minutes after maceration of the tissue. However, the results of various investigations in this laboratory and elsewhere *(5, 7, 8, 9)* have helped to elucidate the nature of the action of this enzyme and explain in part its ability to cause the unusually rapid destruction of pectic substances.[e]

As previously mentioned, the activity of the tomato PG when measured by the rate of glycoside bond breaking (increase in reducing power) was extremely low. It is known that commercial pectinases of this activity do not cause the loss of pectinic substances during the short time that pectinic acids in tomato fruit were destroyed in the "cold break" process. However, a quantitative comparison of the changes in the colloidal properties of pectic acid during the action of mold PG and tomato PG demonstrated a noteworthy difference in their mode of action. It was found that, while calcium-pectate-forming-ability decreases relatively slowly during the action of mold PG and persists until 40 to 60 percent of the theoretical increase in reducing power had occurred, the calcium-pectate-forming-ability vanishes rather abruptly during the action of tomato PG when only 10 to 20 percent of the theoretical reducing power increase had been reached. The increase in reducing power also appears to cease at about 50 percent of the theoretically possible value. On the other hand, whereas the 70 percent ethanol precipitability of the pectic acid substrate disappears entirely during the action of mold PG, the action of tomato PG results only in the loss of the gel-like property of the precipitate. A fine powdery 70 percent neutral ethanol precipitate is obtained from reaction mixtures of pectic acid and tomato PG even after a maximum value of about 50 percent increase in reducing power has been reached. This ethanol precipitated material can be further hydrolyzed by the action of the mold PG to galacturonic acid, and preliminary comparisons of the change in reducing power produced by the mold PG indicate that the material has an average molecular weight corresponding to a pentagalacturonide. It therefore appears that the action of mold PG causes a relatively slow random hydrolysis of pectic acids to the monomer galacturonic acid while the action of the tomato enzyme is to produce a rapid specific depolymerization of the pectic acids to low molecular weight oligo-galacturonides. For this reason it is being suggested that the tomato enzyme be called a pectic acid-depolymerase (DP). This term will be used in the remainder of this paper. As the name implies the true substrate of DP appears to be pectic acid as was shown in the case of tomato DP which had been freed from PM by electrophoresis *(7)*.

It would thus appear that while the mold PG probably produces a more or less random scission of the glycoside bonds of pectic acid, the action of the tomato DP is such as to cause a maximum decrease in molecular weight for a minimum number of glycoside bonds split. Whether this mode of hydrolysis is a characteristic of the enzyme or whether it depends upon the presence of uniquely labile glycoside bonds in the pectic acid molecule is not yet clear. Other evidence such as the ease with which pectin and pectic acids can be degraded by mild heating in solution and by the action of ascorbic acid point toward the latter possibility.

The presence of such bonds might be explained in a number of ways. It might be that branched chain structures are present as in the case of amylose vs. amylopectin; or the $(G_m)_n$ structure, which has been suggested *(3)*, might be involved in which the action of the enzyme was to break the colloidal $(G_m)_n$ complex into the non-colloidal (G_m) unit. Another possibility is that some of the galacturonic acid units in the pectic acid chain are in the furanose rather than in the pyranose form due to lactonization resulting from "kinking" of the chain. The glycoside bonds at these points might be expected to be more labile to hydrolysis and oxidation. Some preliminary evidence indicates the latter hypothesis.

A practical application which might be made of properties of the tomato DP lies in the field of fruit juice clarification. The pectic substances present in most fruit juices tend to stabilize suspensions of insoluble material causing turbidity of the juice. Hydrolysis of the pectic substances "breaks" the suspension and the materials causing turbidity "flock" out resulting in a clear juice. Pithawala, Savur and Sreenivasan *(10)* have recently published some results of the application of the tomato PM-DP complex in the clarification of fruit juice with good results. They obtained good clarification of orange juice in 24 hours with their preparation. These results are particularly encouraging since their preparation was obtained by extracting tomato pulp with phosphate buffer at a slightly alkaline pH. We have found that this procedure results in fairly rapid inactivation of the tomato DP. A highly active DP along with PM is obtained by extracting the pulp with saturated NaCl solution at the pH of the mixture which is about 5.0. Because of the rapidity with which this complex is capable of depolymerizing pectic substances and the cheapness of the source (tomato pulp), further work along these lines may result in a more economical method of fruit juice clarification.

In the course of further work on the tomato DP, it was found that the activity of the enzyme in strong salt solutions was very resistant to heating *(8)*. Saturated NaCl extracts of the enzyme from tomato macerate were found to retain as much as 20 percent of the original activity after standing in a boiling water bath for as long as one hour. This property of the enzyme is rather remarkable inasmuch as the active material present, after heating and centrifuging out the coagulated proteins, does not seem to be a protein itself and does not

[e] Some of the information given in this paper is based on experimental evidence which is to be published in detail in the near future.

appear to be inactivated by treatment with proteolytic enzymes. We are, therefore, in some doubt as to whether the active agent is an enzyme in the accepted sense of the word. This heated DP retains all the properties of the unheated enzyme except with respect to amount of activity.

The importance of this information to the art of processing tomato products should perhaps not be underestimated. Since the DP does not break down highly methylated pectin, the necessity of inactivating PM during the processing is upheld and its inactivation will largely block any action possible by the tomato DP. On the other hand, care should be exercised in the processing to prevent exposure of the product to very high temperatures for relatively long periods of time as this may result in some heat-demethylation of the pectinic acids rendering them subject to later breakdown by the action of the portion of the heat resistant DP which survives. In this laboratory we have already been able to find DP activity present in some commercial canned tomato juices. Some of these juices have a relatively good consistency and possess a good percentage of pectinic acids of a high degree of methylation. The majority of such juices, however, have a poor watery consistency and are practically devoid of pectinic acids or, if obtained shortly after processing, have a small amount of pectinic acids or a very low degree of methylation. These observations seems to uphold the above statements.

In summary, it now appears that the changes leading to the loss of pectinic substances in processed tomato products occur as the result of the cooperative action of tomato PM and DP. The action of PM alone does not in itself lead to an appreciable loss of pectinic acids; however, it is necessary that the pectinic acids be demethylated by the action of PM before the action of the tomato DP may take place. The DP then rapidly depolymerizes the pectic acid substrate made available to units approaching penta-galacturonic acid. The resulting lack of pectic substances is responsible for a loss of colloidal properties and thus for poor consistency of the product.

REFERENCES

1. Kertesz, Z. I., and Loconti, J. D. Factors determining the consistency of commercial canned tomato juice. N. Y. State Agr. Exp. Sta. *Bull.*, **272** (1944).
2. Kertesz, Z. I. Pectic Enzymes II. Pectic enzymes of tomatoes. *Food Research*, **3**, 481 (1938).
3. Kertesz, Z. I. Pectic Enzymes IV. Structural considerations in connection with the enzymic hydrolysis of pectins. *J. Am. Chem. Soc.*, **61**, 2544 (1939).
4. Lineweaver, Hans and Ballou, Gerald A. The effect of cations on the activity of alfalfa pectinesterase. *Arch. Biochem.*, **6**, 373 (1945).
5. MacDonnell, L. R., Jansen, Eugene F., and Lineweaver, Hans. The properties of orange pectinesterase. *Arch. Biochem.*, **6**, 389 (1945).
6. McColloch, R. J., and Kertesz, Z. I. Pectic Enzymes VIII. A comparison of fungal pectin-methylesterase with that of higher plants, especially tomatoes. *Arch. Biochem.*, **13**, 217 (1947).
7. McColloch, R. J. "An Electrophoretic Investigation of Some Pectic Enzymes." Ph.D. dissertation, Kansas State College, Manhattan, Kansas (1948).
8. McColloch, R. J., and Kertesz, Z. I. An unusually heat-resistant pectolytic factor from tomatoes. *Arch. Biochem.*, **17**, 197 (1948).
9. Mottern, H. H., and Hills, Claude H. Low ester pectin from apple pomace. *Ind. Eng. Chem.*, **38**, 1153 (1946).
10. Pithawala, H. R., Savur, G. R., and Sreenivasan, A. Characterization of tomato pectinesterase. *Arch. Biochem.*, **17**, 235 (1948).
11. Smith, H. R. The consistency of tomato catsup. *The Canning Trade*, **54**, No. 1, 14 (1931).
12. Whistler, Roy L., Martin, Albert R., and Harris, Milton. Determination of uronic acids in cellulosic materials. *J. Research Nat. Bur. Standards*, **24**, 13 (1940).
13. Wildman, J. D. Utilization of natural tomato pectin in catsup making. *The Canner*, **72**, No. 1, 11 (1930).

Enzyme Problems in the Citrus Industry[a]

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The Head of the Enzyme Research Division of the U. S. D. A. Bureau of Agricultural and Industrial Chemistry summarizes observations on enzyme research, particularly in reference to citrus fruit. Factors stimulating and inhibiting enzymic changes are discussed and possible economic implications and applications are considered. The need for fundamental research is ably emphasized.

The problems of any industry are always economic ones. But when does an economic problem become an enzyme problem? An economic problem becomes an enzyme problem when somebody makes or loses money by virtue of circumstantial changes in the composition of a raw food product. Such changes are usually reflected in the taste, or "quality"; sometimes in the nutritive value; always in the pocketbook. The responsible enzymes come from two sources; they are inherent in the tissues themselves and they also come from infecting microorganisms.

If we paint the picture with a big brush, it will appear that enzyme action is mainly responsible for three things about a fruit: For its growth, its ripening, and its decay (or shall we be euphemistic and say its spoilage). Each of these phases presents a series of enzyme problems which are in fact very practical agricultural questions. Every day we face the question of what to do if even slight interference is imposed upon one of these processes. Stunted growth, incomplete ripening and premature decay are everyday occurrences. The *reason* therefor may be frost or drought or a host of other things. The *cause* is decidedly an inside job, the failure of something in the plant to do as it should. The *effect* is often all we see, and usually we don't approve of it. The positive effects seen as decay are owing to appropriate enzyme actions; but in growth and ripening the failure of a positive effect is usually the result of enzyme in action—to the lack of appropriate enzyme action. This is plain if we think of what an enzyme does, or

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rather what an enzyme system does. For while we study individual enzyme proteins in the test tube, in real life enzymes occur in groups or systems, and it is the net result of the system's action that we usually see. If one enzyme in a system is destroyed, the system will not function normally. But it is a mistake to suppose that everything would stop. The other enzymes of the group would still continue to act, so far as they could, but the result would create an abnormal state. When a step-wise enzyme process (and most of them are step-wise) is halted in the middle, mid-products rather than end-products accumulate. Mid-products of fruit metabolism are often cell poisons, and often have a bad taste. They contribute importantly to spoilage.

At this point I think we should stop to consider our concept of enzyme. When we define an enzyme as a catalyst we are prone to ignore the most important thing about it, namely what it accomplishes. You will remember the old illustration of an enzyme: It is like a shotgun at a wedding. This is true; but generations of students have got into the habit of thinking that the shotgun is aimed at the preacher. Now I insist that the most important result of the aforesaid weapon is not speeding up the ceremony, but getting the couple married.

Certainly, an enzyme is a catalyst. That is how it functions. But what it really does is to determine down which of several paths energy shall flow. It furnishes a plan which most of the molecules follow in arranging themselves into new compounds. Enzymes create order out of disorder among the countless molecules in a cell. Otherwise, the reactions in a cell would be at random, and the results would be chaotic. An enzyme acts as a sort of guide, and in doing so it arranges things in the order that we call normal development. Enzymes put the reacting groups in the proper order by virtue of their property of specificity. Thus the *specificity* of an enzyme should be more interesting to us than the fact that it is a catalyst. "Enzymes," wrote J. B. S. Haldane, "do not merely accelerate metabolic processes, they determine their direction."

I have been at some pains to point out the fundamental importance of enzyme action in determining the value of agricultural products. Later, I will point out some practical applications. All in all, however, these applications are of minor importance compared to what they might be. The reason for this is our downright ignorance of the fundamental reactions that go on in the growth, ripening and decay of agricultural products, as opposed to our much greater information concerning the corresponding reactions in human tissues. The application of a body of scientific knowledge such as enzyme chemistry to an industry requires first of all that the knowledge itself be in existence. Thereafter, its application is reasonably certain, as things go in this world. I think it is particularly true and very creditable of the food industries that during the war they applied most of their backlog of scientific knowledge. But now they have used it up. For practical applications to continue, it seems likely that the reservoir of scientific knowledge must be refilled. The wisest course to pursue is therefore to create a new supply of fundamental knowledge, in the firm belief that when it is developed, the technologist will find a way to use it. Unfortunately, no one can ever predict when a particular scientific fact is going to become the keystone of a new technical achievement. So this proposed method of solving problems is slow, but science knows no other. It works. The alternative is intuition. I admit intuition sometimes pays off, and quickly; but on the whole it costs more than the scientific method.

Let us now consider the three places where enzyme actions become of importance to the citrus industry; namely in growth, in ripening and in spoilage.

As for growth, the metabolism of the whole tree determines growth, and it presents a chemical picture too complicated for enzyme studies at present. There is, no doubt, a chemical slant to what makes one orange grow large and another small. Is there perhaps some hormone-like substance that starts the growth period sooner than the plant is ready for it? Acetylene, I am told, can do that to pineapples. Maybe we are accumulating something that affects growth in our irrigated soil, or in our modern atmosphere. Or do some of our new insecticides have unsuspected metabolic properties? I'm afraid we are a long way from knowing.

Ripeness is largely determined by the metabolism of the fruit itself; for you can ripen some fruits that are picked green. This problem is then localized, and therefore simpler.

Spoilage is the simplest of all. That obviously involves the fruit alone, sometimes only the "juice." Both ripening and spoilage, however, present many problems to your industry.

For example: What is a ripe orange? Or how nearly ripe is a particular orange? I understand there are some laws on the subject, but there seems to be very little modern information. Both the laws and the information can be easily upset by feeding the tree a trace of arsenic. I wonder if the arsenic changes all the acid to sugar, or if it prevents any sugar changing to acid. The case is probably not very important, but I shouldn't be surprised if the principle involved is quite so.

Frankly, there seems to be no good test for the degree of ripeness of an orange; though it might not be so difficult to find one. It is the sort of problem that is often solved through enzyme tests. For instance, the animal physiologists are now trying to correlate enzyme tests with age in males and with pregnancy in females. Some plant physiologists believe they have correlated the frost-resistance of vegetables and the malting quality of grains with other enzyme tests. The prognosis for an enzyme test of ripeness is favorable.

There are many examples of enzyme problems concerning spoilage. Frost injury, or even mechanical injury to an orange causes the deposition of hesperidin crystals at the site of the injury. Changes in respiration rate also occur. These may not be important observations in themselves, but again, the underlying reasons may be very important to the shipper of fresh fruit. Then there are the spoilages that take place in pasteurized citrus juices. Let me remind you of two of them.

One form of spoilage occurs when the nice looking hazy cloud disintegrates into a sediment. This is perhaps the only enzyme problem in the citrus field that

has been completely solved. As you know, Mr. Stevens of the California Fruit Growers Exchange and Dr. Lineweaver and his group at the Western Regional Research Laboratory contributed heavily to the solution: Simply that some pectin esterase survived the pasteurization. Later with plenty of time to work, it decomposed the pectin into pectic acid. The pectic acid, finding an abundance of calcium in the vicinity, is precipitated as calcium pectate, hence the sediment.

Another form of spoilage in pasteurized juice is the development of off-flavors. It is safe to say they are not all due to the same cause; it is not safe to say much more. In some cases (one of which I will refer to again) there is no present evidence that an enzyme action is involved; in others, it seems likely that there is. Perhaps simultaneous oxidations and reductions in citrus juice are due to residual enzymes that have escaped pasteurization as pectin esterase did. Or perhaps a type of rancidity is started by the heat treatment, and progresses autocatalytically, as rancidity in oils is known to progress. Or perhaps again, residual living bacteria later fabricate a new supply of enzymes in the juice.[c]

I would like to speak now about some of our own work. In doing so, please remember that I am only reporting to you work that has been done by some of my colleagues: Alphabetically speaking, Dr. Axelrod, Dr. Emerson, Dr. Hale, Mr. Jansen and Dr. Nutting.

The subject of *ripening* has engaged most of our attention. As yet the overall picture rather than the individual enzyme systems involved must be studied, because the latter are not known. Could we isolate an enzyme system definitely involved in ripening, we would have a most valuable clue. This much progress has however been made. We know of substances that accelerate the action of the ripening enzymes. The most used is ethylene (whose effect in ripening citrus fruit was observed over 20 years ago by F. E. Denny in this Bureau's Fruit and Vegetable Chemistry Laboratory at Los Angeles). Workers everywhere have studied the accelerating effect of ethylene on ripening in the hope that they might find a clue to the more valuable knowledge of how it works. These overall studies have not proved a total loss. Let me describe one case where they proved very useful.

When we speak of off-flavors that develop in orange juice we usually mean after a period of storage. There is, as you know, an outstanding exception to this. In the juice of the California Navel orange a more or less intense bitter taste develops in an hour or so on standing at room temperature, and immediately on pasteurization. Dr. Emerson in our laboratory undertook an investigation of this.

We soon confirmed the observations of many previous workers that in bitter Navel juice there is a substance known as limonin. It is also present and to a much greater extent in the peel of Navels; but there is much less, if any, in the juice of Valencias except when these are very immature. (However, limonin is found in abundance in the seeds of all sorts of citrus fruits.)[d]

It is evident that any understanding of the genesis of these bitter substances will require a detailed knowledge of their chemistry and chemical properties. It is also obvious that the bitter principles are not present in Navel juice to begin with, so they must be formed there. Therefore, the bitter material has a non-bitter precursor, as the following simple experiment illustrates:

If freshly made Navel juice is immediately extracted with benzene, in which limonin and nomilin are quite soluble, the extract (after removing the benzene, of course) is not bitter. Four hours later, however, if a second extraction is made, the extract is extremely bitter. But several hours later still, a third extract is only slightly bitter. Evidently, the reaction was nearly completed at the time of the second extraction, and had hardly started at the time of the first.

Is there anything about limonin and nomilin that would lead us to suppose they could act this way? For a long time limonin has been known to contain two lactone groups, and nomilin was found to be similar. Higby of the California Fruit Growers Exchange first suggested that perhaps a bitter lactone was formed in the highly acid juice by the lactonization of a hydroxy acid analogous to limonin. Limonin readily dissolves in alkali as a dibasic acid. This solution is not bitter at all. We followed the course of its lactonization to limonin when the diacid was acidified. The lactone form is very bitter. We also found that if the disodium salt of limonin is added to Valencia orange juice, and thus finds itself in an acid medium, the Valencia juice turns bitter. Furthermore, after the addition of an amount of the salt of limonic acid that one would expect to find in Navel juice, the Valencia juice turns bitter at roughly the same rate as does Navel juice. On this evidence it seems reasonable to conclude that limonin makes up the bulk of the material responsible for the bitter taste of Navel juice. (Nomilin has not been found in Navel juice). There seems to be no reason to involve an enzyme in this reaction, but that is far from proof that one is not involved.[e]

The foregoing experiment does not tell us the exact nature of the non-bitter precursor, but it indicates its probable nature, namely a hydroxy acid which contains the limonin (or maybe the nomilin) skelton. The real precursor could just as well be a limonin monolactone monoacid or perhaps a glycoside. If it turns out to be the latter, there is probably a preliminary enzyme hydrolysis to a limonic acid. The course of further work on the formation of the bitter substance may have to wait until this is decided. The question is important

[c] These are not all my own guesses. They came out in many discussions with some of the ablest men in the citrus business. I am specially indebted to Mr. C. P. Wilson, Mr. E. F. Draper and Mr. William Platt. But with their permission, I still insist they are guesses.

[d] In the course of his study, Dr. Emerson also isolated a hitherto unknown bitter principle from citrus (including Navel oranges) which he found to be chemically rather similar to limonin. He named the new substance "nomilin," by right of discovery. The name was derived by rearranging the letters of "limonin." This scheme is frequently employed to name a new substance similar to one already known.

[e] Since this was written some evidence has indicated that the formation of bitter in natural Navel juice is more rapid than in the "dosed" Valencia juice. This may indicate the presence of another precursor of the lactone than the simple hydroxy acid, or a converting catalyst, or (most likely) both.

because either the precursor or an enzyme must be in the solid tissue. If, as seems likely, only acid is needed to turn the precursor bitter, it cannot very well be present in liquid contained in the juice sacs of the orange, or it would have already turned bitter there by virtue of the acidity of the juice. One must conclude then that the precursor exists in the solid portions of the fruit and finds its way from there into the juice.

In the meantime, however, it may be profitable to consider why the precursor of limonin often exists in mature Navel oranges, and not in Valencias. Perhaps it is an abnormality.

I have already pointed out that the difficulty with abnormal metabolism in a fruit is more often the result of incomplete enzyme action, than of too much. We may then speculate, if we wish, that a Navel orange is usually deficient in an enzyme system which a Valencia orange contains. If this is the case, the missing enzyme system must finally develop during the last stages of ripening, for it is well known that very ripe Navels give less bitter juice, and frequently their juice does not turn bitter at all.[f] Under these circumstances one naturally thinks of attempting to stimulate the enzyme action that takes place during ripening, and the simplest method we have is with ethylene. Ethylene may or may not ripen the juicy part of an orange (I would rather not commit myself), but it undoubtedly stimulates ripening in the peel.

Accordingly, we ripened some Navel oranges very thoroughly with ethylene in the laboratory. The juice of the control oranges turnred very bitter, while the juice of the treated oranges no longer did so. The latter juice was musty; and tasted just as though the oranges had been stored in a desiccator (as indeed they were), but it was definitely not bitter. This may not turn out to be a practical way of handling Navel oranges, but the experiment establishes a very interesting point. On the practical side, our next effort will be to attempt to produce ethylene stimulation while the fruit is on the tree, where it may not turn as musty as it does in desiccators. Thanks to the help of Dean Hutchison and Dr. Bachelor of the University of Californria, we hope to try that this fall.[g]

The point at present, however, is that there is evidence that the bitter taste of Navel orange juice is due to the failure of some factor during the usual ripening period of the orange. This factor apparently does exist in very ripe Navels and also in relatively green Valencias, for our experiments showed that only the juice of very young Valencia fruit turned bitter. Furthermore, it must be remembered that the navel of an orange is really an undeveloped, if not actually immature, small orange within the larger orange. So that even at a relatively late stage of development, a Navel orange probably has an unripe spot in it. But what the words "very ripe" and "relatively late" mean in terms of actual maturity, I cannot say. (In a way this illustrates our need for an adequate test of maturity.)

The stimulation of ripening by such reagents as ethylene furnishes an excellent method of studying this phase of metabolism. Logically, however, the reverse method should be equally valuable, namely to observe what would happen if an enzyme system involved in ripening were to be inactivated. Last spring we did not know of any specific inhibitory substances that could be applied to ripe oranges. To obtain this knowledge, we studied the inhibition of several of the known enzymes in ripe oranges, and in the course of that study we had quite an adventure. It turned out that some of the new insecticides developed during the war are tremendously powerful inhibitors of one enzyme that is found in orange flavedo.

You have probably all heard of a new and very efficient war gas affectionately called D. F. P., whose real name is diisopropyl fluorophosphate. This substance, because of its potential danger in war, has been carefully studied by the War Department. It now appears that its poisonous effects are owing to its ability, in very minute quantities, to inactivate an enzyme in the animal body known as choline esterase. Choline esterase hydrolyzes acetylcholine, and is necessary for the proper function of the motor nerves, because the continual change of choline to acetylcholine and then back again to choline is what happens when impulses are started at the beginning of a nerve, and also when they finish up at the end of the nerve. So the effect of D.F.P. is to cause a continuous accumulation of acetylcholine, which eventually leads to paralysis of the nerves involved.

It is known that also in citrus tissue there exists an enzyme that hydrolyzes acetylcholine. (We also found one in wheat germ.) This enzyme in citrus and in wheat germ has been named acetylesterase because it splits many acetic (and propionic) acid esters, including the fat-like triacetin and also acetylcholine. One must not confuse this plant enzyme with the choline esterase of animals; the resemblance is probably only superficial. Nevertheless, Mr. Jansen and Dr. Nutting of this Laboratory found that plant acetylesterase is inhibited by very small concentrations of D. F. P. Furthermore, after a fairly wide search, this is the only plant enzyme so far found to be inhibited by similar minute quantities of the war gas. It looks as though we have a specific inhibitor of citrus acetylesterase. If a solution of the enzyme, prepared from orange flavedo, is used in the test tube, a concentration of 5×10^{-5} Molar of D. F.P. (less than 10 parts per million by weight of the solution) is all that is needed to reduce the activity of the acetylesterase by 50 percent.

The quantity is so small that it was still more surprising to find that another organic phosphate was 50 times more powerful. This material is hexaethyl tetraphosphate (H. E. T. P.), now being used as an insecticide. It is not regarded as a pure substance, but as a mixture of tetraethyl pyrophosphate and ethyl metaphosphate. Both of these constituents are inhibitory, the pyrophosphate somewhat more and the metaphos-

[f] One of the most disconcerting phases of this problem arises from the circumstance that every few years the Navel crop does not give bitter juice. In such years, experiments are sadly limited by the absence of controls. The crop of 1947-48 was such a one.

[g] This experiment was since made, and was probably unsuccessful. Howover, owing to the abnormal season, none of the oranges gave positively bitter juice.

phate somewhat less than the usual mixture. Under the conditions of our experiments, which you must remember are still in the test tube, only two parts of H. E. T.P. in ten million of solution are required to inhibit half of the acetylesterase present.

As soon as this was discovered it seemed a very good idea to expose some live ripe oranges to a trace of H. E. T. P. (the insecticide) and see what happened to the acetylesterase in the live flavedo. This we did, and to our amazement, absolutely nothing happened to the enzyme.

While we worked around trying to find the mistake, some of our inhibited enzyme extracts were quietly resting in the refrigerattor. They rested there for about two weeks, when we tested them again. We then discovered that quite a lot of the once inhibited enzyme had reappeared. To make a long story short, there was something else in our extract of orange flavedo that gradually caused a reversal of this inhibition. No wonder a live orange wasn't noticeably affected.

The regenerative substance seems to have a rough pH range of optimal action, between pH 5 and 6; it is no longer present after a preparation has been heated near boiling. In these respects it acts as an enzyme usually does, though that is still far from proof that it is an enzyme. There is also some evidence that the regenerating substance occurs to the greatest extent in the ordinary "juice" while the acetylesterase occurs mostly in the flavedo. The living orange thus appears to contain within itself the means of protecting this enzyme from destruction, and the mechanism of the means may itself be enzymatic.

On the other hand, the inhibition of acetylesterase in oranges by D. F. P. (the war gas) can be easily demonstrated in the live fruit. Nevertheless, the fruit recovers somewhat if given time. The extracts however show no recovery, as they did after treatment with H. E. T. P. (the insecticide).

I have now tried to summarize for you our ideas of the need for enzyme research in agriculture, using the citrus field as an example. I have indicated some of the problems in that field that we think might be solved or helped to solution by enzyme investigations. Finally, I've told you of two of the recent adventures in our little Enzyme Research Laboratory: The Case of the Bitter Navels, and the Case of the Disappearing Inhibitor. To repeat, I believe that many problems of the citrus industry can be materially helped by fundamental enzyme studies. The scientific method is very slow—often too slow for the patience of industry. But it differs from most things in the world today; ultimately it is reasonably certain.

Further Studies on Frozen Fruit and Vegetable Purees

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This article surveys regulations and standards pertaining to the canned baby-food industry, reports studies of several methods of preparing frozen fruit and vegetable purees, discusses danger of contamination by pathogenic organisms, includes results of a preliminary survey of acceptability to hospital patients of frozen as compared with canned purees.

Introduction

The suggestion of freezing purees for use as baby foods, in certain restricted diets for adults, and in other ways has created considerable interest during the past several years. The investigation in this laboratory on this problem was initiated in about 1940 by M. A. Joslyn and has been continued by the present authors and others. Reports of some phases have already been published by Hohl *(12)* and by Hohl and Smith *(13)*. Many uses for frozen purees other than those mentioned above have been suggested (see Paul, *18*, and Joslyn and Hohl, *14*).

In considering the possibilities of freezing purees several problems are involved. Those upon which data are presented in this paper are the following: What are the chief advantages of frozen over canned purees? What assurance does the consumer have of the microbiological safety of the product? How are the vitamins and other nutrients retained? What would be the best methods of processing, thawing, utilizing and serving?

Standards

A survey was made of all possible sources of regulations and standards used as guides in the manufacture of canned baby foods. The results of this survey may be summarized with the statement that baby foods are manufactured under the general code of the Federal Pure Food, Drug, and Cosmetic Act (Chapter IV, Revision No. 2, February 15, 1946). Private communications with the U. S. Department of Agriculture Production and Marketing Administration, the Food and Drug Administration, and several nationally known canners' associations, and baby food canners, all indicated that the manufacture of purees was governed not only by the "letter" but also by the "spirit of the law." Most processors set themselves rigid and high specifications in the production of baby foods and are alert to possible improvements in their procedures. The administration of the regulations is reasonable. If the processor is using sound, wholesome raw material, under sanitary conditions without adulteration, and is striving for high quality, the requirements of the law are considered to be fulfilled.

So far the only standards established for purees are those for tomato puree, which consist of maximum tolerances for mold filaments and worm fragments, total solids, and adulteration.

The general pediatric literature was searched for data concerning the salt and sugar requirements of infants. The consensus of opinion seems to be that salt may be included in vegetable purees, mainly to increase palatability, since milk supplies all the requirements for salt. Sweetening agents may be added to fruits to increase both their nutritive value and palatability. Infant foods should not, however, be very sweet in order not to encourage an excessive taste for sweets in the child.

Sucrose is usually considered to be suitable, although dextrose, maltose, lactose, or dextrin mixtures are considered advisable in some cases.

Analytical Methods Used in These Studies

Total solids, alcohol-insoluble solids, alcohol-soluble solids, and total acids were determined by the Association of Official Agricultural Chemists *(2)* methods. Vacuum was measured by puncturing the sealed cans with a U. S. Gauge Co. combination vacuum and pressure gauge. pH was measured with a Beckman glass electrode. Flavor and aroma were judged subjectively by several judges. Color was matched with the plates in Maerz and Paul *(16)*, and descriptive names were added as a general guide. Consistency was measured in a Bostwick consistometer, and was recorded as the distance (in centimeters) of flow which occurred within 10 seconds at room temperature (70° ± 5° F.). Sugars were estimated by refractometer and were also sometimes determined chemically by Hassid's ceric sulfate method *(11)*. Sodium chloride was determined by titration with silver nitrate using the procedure described by Vaughn et al. *(28)*. Following essentially the method of Bessey and King *(7)*, ascorbic acid was determined by direct titration of a metaphosphoric acid extract. Thiamin was determined by the fluorometric procedure of Conner and Straub *(9)* and riboflavin by Mackinney and Sūgihara's *(15)* modification of the Conner and Straub combined method for these two vitamins. Vitamin A, or more accurately, total carotinoid, was determined by the following procedure outlined by Mackinney of this laboratory. A 5- to 50-gram sample was extracted by grinding with sand and acetone in a mortar until colorless, using successive portions of acetone; 25 cc. of petroleum ether were then added to the acetone extract with a separatory funnel, the contents gently shaken, washed with water (using a scrubbing technique developed by Dr. Mackinney) and the aqueous layer discarded. This was repeated. If chlorophyll was present the extract was at first prepared with alkaline methyl alcohol to saponify the chlorophyll, which was then removed by shaking with water. A suitable aliquot was chromatographed using a firmly packed column of MgO and Supercel (1:1 ratio by weight). The photoelectric determination of carotene was essentially that of A. O. A. C. *(2)*, sections 36.10 and 36.15, using the Evelyn colorimeter.

Comparative Analyses of Canned and Frozen Purees

A selection was made of representative samples of commercially canned and experimentally frozen fruit and vegetable purees prepared in a variety of ways. These were analyzed chemically and compared for general appearance, odor, palatability and consistency.

The results of these analyses are summarized in Tables 1 and 2. A study of these tables shows that for fruits the flavor and aroma of frozen puree are not always superior to those of canned puree. In vegetable purees the difference between canned and frozen aroma and flavor is so great that the relative merits of the two types become a matter of individual taste. In color, the results varied so greatly with brand, and probably with variety of fruit, that no general comparison could be made between canned and frozen samples in this regard. In green vegetables, the color of frozen purees is far more natural than the yellow-green which is typical of all canned green vegetables. The pH and total acidities showed no consistent differences between standard canned samples and our experimentally frozen ones. Sugar (and consequently also total solids) content of our frozen samples was higher than that of the canned, indicating that the 5 + 1 ratio which we used

TABLE 1

Summary of Observations on Flavor, Aroma, Color and Consistency of Experimentally Frozen and Commercially Canned Baby Foods

Sample	No. of Cans	Aroma	Flavor	Color (Maerz and Paul)	Consistency, flow in cm. in 10 secs.
Canned Apple Sauce	4	Good	Good	Variable	4.1
Frozen Apple Sauce	4	Fair	Fair	Variable	2.2
Canned Apple and Prune	1	Fair	Good	8 L 10 (Dark brown)	5.5
Frozen Apple and Prune					
Canned Apricot and Peach	1	Good	Good	10 L 10 (Orange peel)	7.3
Frozen Apricot and Peach	1	Fair	Good	9 L 9 (Lighter Orange	11.5
Frozen Apricot and Apple	1	Fair	Good	10 L 8 (Florida Gold)	5.0
Frozen Nectarine and Plum	1	Good	Good	11 J 7 (Greenish Apricot)	7.8
Canned Peach	4	Good	Good	Variable	9.4
Frozen Peach	2	Fair	Fair	Variable	13.5
Canned Peach, Pear, Apricot	1	Stale	Good	10 L 8 (Florida Gold)	7.5
Vegetable Soup	4	Good	Fair	Variable	1.5
Canned Beets	2	None	Fair	Good	2.5
Frozen Beets	1	Good	Good	Good	0
Canned Carrots	1	Good	Good	9 L 10 (Marigold)	4.5
Frozen Carrots	1	Good	Good	2 A 12	0
Canned Beans (green)	1	Good	Flat	21 L 1 (Yellow-green)	4.5
Canned Peas	4	Good	Fair	Varied (Yellow-green)	0.6
Frozen Peas	1	Heavy	Starchy	18 K 7 (Bright spring green)	0
Canned Spinach	4	Good	Good	15 L 5 (Olive green)	2.0
Frozen Spinach	2	Strong	Immature	Excellent	very runny

TABLE 2

Summary of Chemical Analyses of Experimentally Frozen and Commercially Canned Baby Foods

Sample	No. of Samples	Total Solids, percent	Alcohol Soluble Solids, percent	Alcohol Insoluble Solids, percent	Total [1] Acid	Ascorbic Acid, Mg./100g.	pH	Total Soluble Solids by Refractometer, percent	Salt (NaCl), percent
Canned Apple Sauce	4	15.4	....	2.8	0.38	0	3.5	14.2	....
Frozen Apple Sauce	4	25.3	20.1	2.6	0.38	0	3.4	22.8	....
Canned Apple and Prune	1	26.6	....	4.3	0.69	0	3.7	....	....
Canned Apricot and Peach	1	8.4	....	3.3	0.80	2	4.0	....	....
Frozen Apricot and Peach	1	24.3	22.8	2.1	0.61	33.1	4.2	23.7	....
Frozen Apricot and Apple	1	25.0	21.6	3.5	0.63	0	4.0	23.7	....
Frozen Nectarine and Plum	1	30.2	25.4	2.5	0.76	16.0	3.9	29.2	....
Canned Peach	4	15.7	....	2.0	0.48	0	3.7	14.8	....
Frozen Peach	2	21.3	19.6	1.7	0.45	15.7	4.1	21.0	....
Canned Peach, Pear, Apricot	1	19.1	....	3.2	0.61	2	3.9	....	....
Vegetable Soup	4	12.3	....	7.7	0.18	1.8	5.1 Ave.	....	....
Canned Beets	2	16.5	....	....	0.42	0	4.8	....	0.72
Frozen Beets	1	16.4	....	4.2	0.18	trace	5.9	....	0
Canned Carrots	1	8.9	....	3.1	0.13	4.5	4.9	....	0
Frozen Carrots	1	16.1	....	4.5	0.24	0	6.0	....	0
Canned Beans (green)	1	8.5	....	4.6	0.29	1	5.2	....	0.72
Canned Peas	4	15.2	....	11.6	0.19	7.6	5.8	....	0.48
Frozen Peas	1	21.6	....	15.7	0.32	15.8	6.9	....	....
Canned Spinach	4	8.8	....	5.0	0.15	19.9	5.2	....	0.68
Frozen Spinach	2	8.3	....	5.1	0.20	13.2	6.5	....	0

[1] Total acid is expressed as grams of citric (for peach, nectarine and plum, vegetable soup, beets, and peas) or as grams malic acid (for apples, carrots, spinach, and green beans) per 100 grams of sample.

for many of these samples was higher than that usually used in commercial practice. Ascorbic acid was notably higher in most frozen than in canned fruit and vegetable purees. The most striking difference between the frozen and canned purees was in their consistency. All the frozen samples (except apple sauce, carrots and beets) were much more fluid than similar canned samples even though the total solids contents of the former were usually higher. In such vegetables as spinach and asparagus, the purees tended to separate into a liquid and a solid phase after freezing and thawing. This phenomenon of change in consistency as a result of freezing and thawing of purees is being studied further. It is one of the most troublesome problems still to be solved and is probably related to the problem of texture breakdown in vegetables and fruits frozen in the usual manner. Salt content of commercially canned vegetable purees varied from 0 to 0.92 percent and obviously the quantity added was determined by the individual preference of the manufacturer.

Studies on Methods of Preparation of Purees for Freezing

A selection of well known varieties of peaches and a few numbered seedling varieties, all collected from the University's experimental orchards at Winters, California, at optimum eating maturity, were made into purees by the following procedure: The whole fruit was steamed for one minute, skins were slipped off and bruises trimmed away, fruits were halved and pitted, steamed for four minutes, air-cooled quickly and then pureed in an American Utensil Co. Sep-ro-sieve, using a 0.03 inch diameter screen. The pulp was run through the machine two or three times in order to extract a maximum of solids. Sugar was added at the rate of ten percent by weight and the purees were filled into glass jars, frozen and stored at 0° F. The above procedure made purees of excellent fresh flavor and keeping qualities. This amount of heating did not impart a cooked flavor to the resulting purees, but it did prevent enzymatic oxidative changes during storage. Apricots, nectarines, plums, and fresh prunes prepared in this same general manner, except that the skins were not removed before passing the fruit through the puree machine, also all made excellent products. If blanching was omitted the fruits tended to become oxidized in flavor, and brown on the surfaces.

Apple and pear purees were best prepared by paring, blanching or precooking (for apple sauce) before being passed through the Sep-ro-sieve. Dried prunes were also used for the preparation of purees. The best procedure for this fruit was to soak, and cook the fruit in the usual manner (without added sugar), pit and puree (including the cooking water) and freeze. If prunes of good initial quality are used, such a product is excellent, but it has no marked advantage over a similar canned product. Fresh prunes give a puree of better flavor and higher vitamin content than the dried prunes. However, if commercial production of frozen purees for baby foods ever becomes a reality, the manufacturers have all expressed the belief that a complete line of frozen baby foods would be highly desirable. Successful purees have been prepared in the laboratory from several varieties of peaches, nectarines, apricots, pears, and apples, as well as from figs, guavas, persimmons, and other fruits. For baby food purposes, fruit blends may offer certain advantages such as cutting down the natural tartness or improving the consistency of certain fruits. Prune,

apple or pear purees often have a thick consistency while nectarines, peaches, apricots, etc., are too thin. Thus pear and Fellenberg (Italian) prune, plum and nectarine, apricot and apple, or plum and a bland apple, or figs and more acid fruits made excellent blends.

A series of experiments were made on the effect of various antioxidants, individually or in combination, upon the quality and particularly upon the ascorbic

TABLE 3

Effect of Sulfur, Citric Acid and Blanching Upon Retention of Added Ascorbic Acid in Apricot Purees

	Treatment of Original Sample			Percent of Original Ascorbic Acid Left After			pH	Total Acid (as Grams Malic per 100 g.)	Color, Maerz and Paul
	Initial Ascorbic Acid Added (mg./100 g.)	Added SO_2 (p.p.m.)	Added Citric Acid (percent)	1 mo.	6 mos.	1 yr.			After 1 yr.'s storage
Raw	0	0	0	....	....	....	4.0	0.79	2 A 12
Raw	25	0	0	72	48	28	4.0	0.80	2 A 12
Raw	50	0	0	72	70	70	4.0	0.80	2 A 12
Raw	75	0	0	80	79	....	4.0	0.80	2 A 12
Raw	100	0	0	69	71	....	4.0	0.80	2 A 12
Raw	25	25	0	88	88	86	4.0	0.81	9 L 10
Raw	100	25	0	85	85	77	4.0	0.85	9 L 10
Raw	0	0	0.5	....	....	....	3.6	1.13	2 A 12
Raw	25	0	0.5	60	68	48	3.6	1.12	2 A 12
Raw	50	0	0.5	72	72	64	3.6	1.17	2 A 12
Raw	75	0	0.5	56	56	73	3.6	1.19	9 L 11
Raw	100	0	0.5	87	87	74	3.6	1.20	9 L 11
Raw	0	100	0	....	....	....	3.6	1.15	9 L 10
Blanched	25	0	0	56	56	56	3.9	0.72	9 L 11
Blanched	50	0	0	80	80	74	3.9	0.74	9 L 11
Blanched	75	0	0	76	74	77	3.9	0.74	9 L 11
Blanched	100	0	0	83	80	82	3.9	0.74	9 L 11
Blanched	50	100	0	80	84	100	3.9	0.78	9 L 10

TABLE 4

Effect of Sulfur Dioxide, Citric Acid, and Blanching Upon Retention of Added Ascorbic Acid in Nectarine Purees

	Treatment of Original Sample			Percent of Original Amount Added		pH	Total Acid (as Grams Citric Acid per 100 g.)	Color, Maerz and Paul	
	Ascorbic Acid Added (mg./100 g.)	SO_2 Added (p.p.m.)	Citric Acid Added (percent)	Ascorbic Acid, 4 mos.	Ascorbic Acid, 1 yr.			3 mos. after freezing	14 mos. after freezing
Raw—Gower and New Boy	0	0	0	....	....	4.0	0.62	9 E 7	11 D 7
Raw—Gower and New Boy	50	0	0	100 +	88	3.98	0.67	9 E 7	11 D 7
Raw—Gower and New Boy	100	0	0	100 +	85	3.98	0.68	9 E 7	11 D 7
Raw—Gower and New Boy	50	25	0	100 +	100	3.88	0.68	11 B 8	11 G 7
Raw—Gower and New Boy	100	25	0	100 +	93	3.79	0.69	11 A 8	11 G 7
Raw—Stanwick	0	0	0	....	....	4.45	0.42	10 I 4	10 H 3
Blanched—Stanwick	0	0	0	....	....	4.60	0.39	10 H 4	10 E 2
Blanched—Stanwick	200	0	0	95	58	4.50	0.39	10 H 4	10 H 3
Raw—Stanwick	50	0	0	100 +	100	4.40	0.42	10 I 3	10 I 2
Raw—Stanwick	100	0	0	100 +	91	4.40	0.43	10 I 3	10 H 2
Raw—Stanwick	200	0	0	89	93	4.10	0.50	10 F 3	10 H 2
Raw—Stanwick	50	25	0	100 +	100	4.29	0.44	11 F 2	10 H 2
Raw—Stanwick	100	25	0	100 +	99	4.21	0.45	11 F 2	10 H 2
Raw—Stanwick	200	25	0	86	45	4.15	0.49	11 F 2	10 H 2
Raw—Stanwick	50	0	0.5	84	100	3.65	0.90	11 G 3	10 H 2
Raw—Stanwick	100	0	0.5	66	97	3.67	0.90	11 G 3	10 H 3
Raw—Stanwick	200	0	0.5	77	100 +	3.62	0.98	11 G 3	10 H 2
Raw—Yellow Seedling	0	0	0	....	....	4.09	0.40	10 L 9	9 L 8
Raw—Yellow Seedling	50	0	0	72	78	4.08	0.46	10 L 9	9 L 8
Raw—Yellow Seedling	100	0	0	81	100 +	4.02	0.46	10 L 9	10 L 7
Raw—Yellow Seedling	200	0	0	71	88	3.96	0.47	10 L 9	9 L 8
Raw—Yellow Seedling	50	25	0	100 +	92	4.08	0.45	10 L 8	9 L 7
Raw—Yellow Seedling	100	25	0	84	79	4.05	0.47	10 L 8	9 L 7
Raw—Yellow Seedling	200	25	0	100 +	91	4.00	0.49	10 L 8	9 L 7
Raw—Yellow Seedling	50	0	0.5	100 +	84	3.75	0.94	9 L 8	10 L 8
Raw—Yellow Seedling	100	0	0.5	98	88	3.70	0.94	10 L 9	10 L 8

TABLE 5

Effect of Sulfur Dioxide, Citric Acid, and Blanching Upon Retention of Added Ascorbic Acid in Peach Purees

	Treatment of Original Sample			Percent of Original Amount Added		pH	Total Acid (as Grams Citric/100 g.)	Color, Maerz and Paul	
	Ascorbic Acid Added (mg./100 g.)	SO_2 Added (p.p.m.)	Citric Acid Added (percent)	Ascorbic Acid, 4 mos.	Ascorbic Acid, 1 yr.			3 mos. after freezing	14 mos. after freezing
Raw	0	0	0	....	....	4.0	0.49	13 B 11	13 B 11
Raw	40	0	0	80	57	4.0	0.49	11 L 8	10 L 8
Raw	80	0	0	100 +	76	4.0	0.47	11 K 9	9 L 8
Raw	120	0	0	100 +	83	3.95	0.48	11 L 9	9 L 8
Raw	40	0	.96	75	70	3.35	1.39	11 L 9	10 L 8
Raw	40	25	0	97	80	3.70	0.45	9 L 8	10 L 8
Raw	80	25	0	100 +	90	3.67	0.48	9 L 8	10 L 8
Blanched	0	0	0	....	....	4.05	0.46	11 L 7	10 L 7
Blanched	40	0	.96	70	100	3.40	1.33	11 L 7	10 L 8
Blanched	40	0	0	100	100	4.00	0.50	11 L 8	10 L 8
Raw	100	0	0	91	64	3.95	0.53	10 K 9	10 L 8
Blanched	100	0	0	100	84	3.95	0.53	11 K 9	10 K 7

acid (vitamin C) retention of frozen apricot, nectarine and peach purees. Tables 3, 4, 5, summarize the results of these experiments. A study of the general trends in these three tables indicates that 50 p.p.m. or higher concentrations of ascorbic acid in purees are more completely retained during storage in the raw and blanched series. In general, the blanched purees retained the added ascorbic acid better than the unblanched. Both SO_2 and citric appeared to exert slight protective effect upon ascorbic-acid retention in these purees during storage. The results were rather irregular, however, and more data would be required to prove the point definitely. Some of the fruit purees showed the protective effect while others were almost completely lacking in such trends. In Tables 3, 4, and 5, the total acidities, pH values and colors (taken from Maerz and Paul) are also recorded. For apricot purees the colors were tabulated only for the year-old samples, since those recorded earlier showed no differences, all of them being 2A12. The nectarine and peach samples showed greater changes during storage. The samples having the greatest additions of ascorbic acid and those with supplements of SO_2 showed the least color change during storage.

During the 1946 season varieties shown in Table 6 were tested for their suitability for freezing both as dessert fruit and as purees. All of these purees, except the last one listed, were prepared by pitting and trimming the fruit, steaming the halves for four minutes, slipping off the skins and passing the peeled fruit through the Sep-ro-sieve two or three times. Sugar was added at the rate of ten percent by weight. It will be noted from the data in Table 6 that the varieties differed considerably in color, pH, acidity, ascorbic acid content, flavor, aroma and consistency. Those whose consistometer readings were above 11 or 12 centimeters of flow in 10 seconds were too thin for baby foods. All of these purees were thinner than any of the commercially canned samples tested. Therefore, in the following season a number of varieties of apricots, peaches and nectarines were prepared in several different ways in an attempt to achieve better consistency in the frozen and thawed puree. A portion of each variety was prepared just as described above. Another portion was washed, trimmed, pitted, halved, steamed for four minutes, dehydrated to half its original weight and then made into a puree. Also an apricot puree was made by still another method, namely that of dehydrating to half weight after preparing the puree. This procedure was later abandoned because of greater difficulty in handling. The results of these experiments and the chemical analyses of these samples are summarized in Tables 7 and 8. It may be noted that the consistencies of the purees prepared just as they had been prepared in the previous season were all higher than those prepared from the corresponding varieties in the previous year. This may have been caused in part by seasonal differences—the 1946-1947 winter having been an extraordinarily dry one. It may also be noted that the sweetened purees always were more fluid in consistency than the corresponding unsweetened ones.

A partially concentrated frozen puree may be desirable for the saving in storage space which it would entail. Water could then be added at the time of serving. However, experimentation showed that addition of water in equivalent volume to that removed during dehydration resulted in a much more fluid puree than that which had been made by making puree from the original undehydrated fruit. The following example of unsweetened Rio Oso Gem peach purees illustrates this point: That prepared directly from the blanched fresh peaches flowed 8 centimeters in ten seconds at room temperature. That prepared from the half-dehydrated fruit flowed 5.2 centimeters while the same puree diluted back by addition of an equal weight of water flowed 14.5 centimeters and that diluted with one part water to two of the concentrated puree flowed 9.2 centimeters in ten seconds.

In the preparation of vegetable purees several different procedures were tested. It soon became apparent that the American Utensil Co. juice extractor which served well for fruits was not suitable for the preparation of purees from such vegetables as asparagus, spinch, broccoli, snap beans, nor even carrots or beets. There was not enough pressure or mechanical action to separate the soft tissues from the more or less stringy vascular elements of these leaf, stem, or root tissues. A Rietz disintegrator was successfully used in preparing purees from these vegetables, but this machine permits considerable aeration during preparation of puree. The Langsencamp laboratory pulper was used for some of the experiments and it yielded an excellent product.

TABLE 6

Qualities of Several Varieties of Peach Purees

Variety	Aroma	Flavor	Color (Maerz and Paul)	Consistency (cms. in 10-secs.)	pH	Total Acidity (gms. citric per 100 g.)	Ascorbic Acid (mg./100 g.)
Early Crawford	good	good	10 L 8	11.3	3.9	0.49	3.5
Late Crawford	fair	tart	10 K 8	16.5	3.6	0.61	2
Elberta	fair	watery	11 L 8	24 +	3.8	0.37	0
Early Elberta	heavy	over-ripe	10 L 9	18.0	4.0	0.50	3
Fay Elberta	good	watery	10 K 8	24 +	3.8	0.39	0
July Elberta	fair	too sweet	10 L 8	16.5	3.8	0.44	4
J. H. Hale	fair	good	11 C 10	16.0	3.8	0.43	0
White Hale	good	slightly flat	11 C 4	14.5	3.7	0.54	1
Lovell	fair	good	11 L 7	11.5	3.7	0.56	3
Muir	fair	low in acidity	10 L 7	14.0	4.4	0.33	3
Rio Oso Gem	good	good	11 E 11	17.5	3.8	0.47	0
St. John	good	sweetish	10 L 8	9.5	4.0	0.52	5
Seedling W 24-29	good	watery	10 L 10	15.5	3.8	0.49	3
Seedling W 24-35B	fair	tart	10 L 7	15.5	3.6	0.69	0
Sunbeam	good	watery	10 L 7	18.5	3.9	0.52	0
Raw Sunbeam (1945)	fair	watery	9 L 5	24 +	4.0	0.47	0

TABLE 7

Effect of Partial Dehydration of Fruits on Quality of Fruit Purees

Sample	Treatment	Aroma	Flavor	Color	Consistency (cm. of flow in 10 sec.)
Apricot Royal	fresh	fair	sharp	12 L 10	6.8
	blanched, dehydrated and pureed	very good	good	12 I 12	4.0
	blanched, pureed and dehydrated	good	fair	12 I 12	3.8
	blanched and pureed	good	tart	10 L 11	8
	blanched, dehydrated, pureed and 10% sugar	good	good	10 L 12	6
	blanched, pureed and 10% sugar	good	fair	10 L 12	8.4
Peaches Elberta	fresh	fair	sharp	11 K 8	10.0
	blanched, dehydrated and pureed	good	fair	11 L 11	6.3
	blanched, pureed and 10% sugar	good	tart	11 K 10	11.3
J. H. Hale	fresh	fair	flat	11 K 9	7.5
	blanched, dehydrated and pureed	good	fair	11 L 11	6.6
	blanched, pureed and 10% sugar	good	fair	11 K 10	9.0
Fay Elberta	fresh	fair	ripe	11 J 9	10.3
	blanched, dehydrated, pureed and 5% sugar	weak	fair	10 K 7	9.5
	blanched, pureed and 10% sugar	weak	sweet	10 K 6	17.0
	blanched, pureed and dehydrated	weak	sweet	10 K 7	9.5
Rio Oso Gem	fresh	fair	good	10 J 8	8.9
	blanched, pureed and 10% sugar	weak	good	10 K 9	10.5
	blanched and pureed	weak	sharp	10 K 8	10.5
	blanched, pureed, dehydrated and 10% sugar	weak	slightly sweet	11 L 9	6.5
	blanched, dehydrated and pureed	weak	slightly sharp	11 L 8	4.5
	blanched, dehydrated, pureed and packed with 5% sugar dry on top	weak	good	11 L 9	5.0
Nectarines Philip Seedling 24-6c	fresh	fair	good	11 J 9	6.5
	pureed and 10% sugar	fair	fair	11 J 10	5.8
	blanched, pureed and 10% sugar	good	fair	11 J 9	6.2
Humboldt	fresh	heavy	oxidized	11 L 9	3.0
	blanched, dehydrated and pureed	fair	oxidized	11 L 10	2.6
	blanched, pureed and 10% sugar	fair	oxidized	10 L 8	7.6
Giallo di Padova	blanched, pureed and 10% sugar	weak	sharp	10 L 5	8.2
Stanwick	blanched, dehydrated, pureed and 5% sugar	good	sweet	10 I 2	2.0
	blanched, dehydrated, pureed and packed with 5% sugar dry on top	good	sweet	10 H 2	2.2
	blanched, pureed and 5% sugar	weak	good	10 I 2	7.4

TABLE 8

Effect of Partial Dehydration of Fruits on Composition of Fruit Purees

Sample	Treatment	pH	Total Solids percent	Alcohol Soluble Solids percent	Water and Alcohol Insoluble percent	Total Acid*	Ascorbic Acid mg./100 g.	Carotene mg./100 g.	Sucrose by Refracto-meter percent
Apricot Royal	fresh	3.6	15.6	13.5	....	1.45	0	2000	14
	blanched, dehydrated and pureed	3.8	21.35	23.2	2.16	2.14	7.1	2143	19.4
	blanched, pureed and dehydrated	3.9	21.70	20.7	1.09	2.05	5.2	2287	21.4
	blanched and pureed	3.9	16.21	11.8	1.01	1.74	8.9	2036	14.6
	blanched, dehydrated, pureed and 10% sugar	3.6	25.31	22.9	1.03	1.57	7.5	2000	24.8
	blanched, pureed and 10% sugar	3.6	27.11	....	....	1.37	10.3	1700	22.6
Peaches Elberta	fresh	3.8	13.2	10.8	1.01	.46	0	255	12.3
	blanched, dehydrated and pureed	3.9	25.4	11.1	1.23	.61	1.7	290	24.5
	blanched, pureed and 10% sugar	3.9	21.9	10.0	1.12	.45	0	249	21.2
J. H. Hale	fresh	3.5	13.8	12.1	1.05	.73	0	150	12.7
	blanched, dehydrated and pureed	3.7	26.9	....	5.08	.91	0	173	25.3
	blanched, pureed and 10% sugar	3.7	21.9	11.9	1.10	.65	0	143	20.9
Fay Elberta	fresh	3.8	11.7	9.4	1.00	.55	4.9	1060	10.7
	blanched, dehydrated, pureed and 5% sugar	3.8	22.9	....	....	.71	....	....	22.2
	blanched, pureed and 10% sugar	3.8	20.3	....	....	.47	....	....	22.4
	blanched, pureed and dehydrated	3.8	31.0	....	....	.68	....	....	29.9
Rio Oso Gem	fresh	3.6	13.8	11.6	1.23	.78	6.0	1345	13.0
	blanched, pureed and 10% sugar	3.7	14.5	12.0	1.34	.61	5.5	1365	20.8
	blanched and pureed	3.7	....	....	....	.71	....	....	13.3
	blanched, pureed, dehydrated and 10% sugar	3.7	....	....	....	.87	....	....	25.9
	blanched, dehydrated and pureed	3.7	....	....	....	1.07	....	....	23.2
	blanched, dehydrated, pureed and packed with 5% sugar dry on top	3.7	....	....	....	.98	....	....	32.7
Nectarines Philp Seedling 24-6c	fresh	3.6	18.5	16.0	1.5	.88	15.2	425	17.6
	pureed and 10% sugar	3.6	18.1	15.5	1.01	.82	17.1	550	27.0
	blanched, pureed and 10% sugar	3.6	17.9	15.3	1.2	.86	14.3	500	27.2
Humboldt	fresh	4.1	22.3	18.1	2.2	.66	23.8	1560	21.2
	blanched, dehydrated and pureed	4.0	35.3	16.9	1.9	.79	16.4	1190	34.0
	blanched, pureed and 10% sugar	4.2	28.8	....	....	.56	....	....	27.5
Giallo di Padova	blanched, pureed and 10% sugar	3.7	27.8	....	....	.98	....	....	26.8
Stanwick	blanched, dehydrated, pureed and 5% sugar	4.2	37.1	....	....	.83	....	....	35.1
	blanched, dehydrated, pureed and packed with 5% sugar dry on top	4.2	37.0	....	....	.89	....	....	35.6
	blanched, pureed and 5% sugar	4.3	29.2	....	....	.54	....	....	28.0

* Expressed as grams malic per 100 grams for apricots and as grams citric for peaches and nectarines.

When a suitable puree machine had been chosen for a particular vegetable, comparisons were made among the following procedures for preparation for freezing:

(a) Prepare, blanch and cool the vegetable as for regular freezing, make puree, package and freeze.

(b) Prepare and precook the vegetable as for serving fresh, cool, make puree, package and freeze.

(c) Prepare, blanch and cool (as in *a*), make puree, heat to finish cooking and fill into scrupulously clean (sterile, if possible) containers, cool quickly and freeze.

(d) Partially dehydrate vegetables (to 50 percent moisture), make puree, package and freeze.

In general, the best quality, from the adult taster's point of view, was achieved by using either method *(a)* or *(c)*. Puree prepared by method *(a)* required some cooking after thawing and this may be a bit troublesome at times since purees "set" and scorch easily unless a double boiler is used and if a double boiler is used, it is difficult to accomplish the desired cooking. None of the samples prepared by method *(b)* were as acceptable as those prepared by the other methods. Method *(c)* was introduced in order to minimize the microbial contamination of the product. Most of the samples of green vegetables prepared in that way were better than those prepared by method *(b)*, because the green color and fresh flavor were better retained in that way. If frozen purees were prepared commercially in such a manner and were filled into sterile containers, they could be almost as safe, microbiologically, as the canned product. The partial dehydration method *(d)* was satisfactory for carrot puree, but for green vegetables it had a tendency to darken the color. Table 9 shows the results of such comparative processing methods with carrots.

A few vegetable purees were used to make a comparison of the following ways of preparing baby foods:

(a) Wash, prepare as for freezing, blanch, cool, make puree, package, freeze and store at 0° F.

(b) Prepare puree as for (a), package in sanitary cans, exhaust, seal heat process (8 oz. can, 30 minutes at 240° F.), cool and store at room temperature.

(c) Prepare puree as for (a), heat to finish cooking and fill into *clean* containers. Store at 0° F.

(d) Cook the fresh vegetable and press through a sieve as the housewife would do if she were preparing her own puree.

TABLE 9

Effect of Several Methods of Preparation on Quality and Composition of Carrot Purees

Method of Preparation	Aroma	Flavor	Color Maerz and Paul	Consistency flow in cms. in 10 secs.	Total Solids Percent	Alcohol Insoluble Solids Percent	Total Acid (as grams malic/ 100 g.	pH	Ascorbic Acid mg./100 g.
Blanched, pureed, frozen	Hay-like	Hay-like	11 L 12	0	10.75	3.10	.12	6.22	3.00
Precooked whole, pureed, frozen	Hay-like	Hay-like	11 L 10	0	9.5	....	.12	6.40	2.75
Precooked diced, pureed, frozen	Good	Good	10 L 10	0	12.6	3.76	.12	6.28	....
Blanch, puree, cook, and freeze	Somewhat hay-like	Hay-like	11 L 11	0	12.41	4.00	.13	6.10	2.60
Blanch, dehydrate, puree, and freeze	Hay-like	Hay-like	11 L 11	0	12.40	2.84	.13	6.0	4.29

TABLE 10

Effect of Method of Preparation Upon Quality of Vegetable Purees

Vegetable	Method of Preparation	Aroma	Flavor	Color Maerz and Paul	Consistency flow in cms. in 10 secs.
Snap beans	Raw fresh vegetable	strong	fair	14 L 2	5.5
	"Home cooked" and pureed	good	good	14 L 3	13
	Freshly prepared puree	fresh	half cooked	12 L 2	13
	Frozen puree	good	good	14 L 1	11.9
	Canned puree	canned	canned	13 L 7	11.3
Asparagus	Raw fresh vegetable	fair	slightly astringent	22 K 7	0.5
	Freshly prepared puree	good	good, flat	22 L 4	3.5
	Blanched, pureed, frozen	good	good, flat	22 L 5	5.8
	Canned puree	good	good	21 L 1	2.3
	Blanched, pureed, cooked, frozen	good	poor	22 L 5	5.5
	"Home cooked" and pureed	good	insipid	22 L 3	very fluid
Asparagus (after 6 mos. storage)	Blanched, pureed, frozen	strong	astringent	21 L 5	H_2O separated
	Canned puree	stale	good	12 L 3	1.5
	Blanched, pureed, cooked, frozen	strong	poor	21 L 3	H_2O separated

TABLE 11

Effect of Method of Preparation on Composition of Vegetable Purees

Vegetable	Method of Preparation	Total Solids percent	Alcohol Insoluble percent	pH	Total Acid gm. malic per 100 g.	Ascorbic Acid mg./100 g.	Carotene δ/100 g.	Riboflavin δ/g.	Thiamin δ/g.
Snap beans	Raw fresh vegetable	10.1	1.75	6.0	.38	9.6	120	0.66	0.61
	"Home cooked" and pureed	9.6	1.34	6.05	.31	4.3	120	0.94	0.39
	Freshly prepared puree	9.56	1.45	5.55	.43	9.0	160	1.22	0.30
	Frozen puree	9.39	1.52	5.45	.38	6.0	160	1.11	0.30
	Canned puree	9.50	1.71	5.32	.34	7.7	130	....	0.30
Asparagus	Raw fresh vegetable	7.51	....	6.3	....	25.5	334	1.48	0.52
	Freshly prepared puree	6.76	....	6.1	....	22.4	....	3.32	1.00
	Blanched, pureed, frozen	6.64	....	6.1	....	15.1	....	2.22	0.60
	Canned puree	6.89	....	5.4	....	15.9	....	1.85	0.70
	Blanched, pureed, cooked, frozen	6.98	....	6.1	....	12.6	....	2.58	0.48
	"Home cooked" and pureed	....	....	6.4	....	18.8	....	....	....
Asparagus (after 6 mos. storage)	Blanched, pureed, frozen	6.83	....	6.7	.15	11.3	280	1.17	0.54
	Canned puree	7.01	....	5.7	.23	16.1	380	1.23	0.35
	Blanched, pureed, cooked, frozen	7.11	....	6.6	.19	11.7	270	1.12	0.44

Analyses were made on these as well as on the original fresh material, and on the original purée prepared for *(a)*, *(b)* and *(c)* before it was frozen or further processed. Tables 10 and 11 summarize the results of these experiments on snap bean and asparagus purees. It will be noted that the riboflavin contents of both vegetables, thiamin of asparagus and the carotene of snap beans, were higher in the freshly prepared puree than in the original raw fresh vegetables. Possibly this may be explained by the fact that the vitamin-rich portions of the vegetables were the tender parts which remained in the puree while the fibrous conducting tissues which remained behind had little original vitamin content. Another observation of interest is that the processed purees (both frozen and canned) had a generally higher vitamin content than those prepared from similar raw material by ordinary home methods. (The general method followed to simulate home conditions was boiling the prepared vegetable in enough water to cover it in an open aluminum kettle just long enough to make it tender, pressing it through a stainless steel sieve, and adding back enough of the cooking water to give the desired consistency.)

When the frozen and canned purees of these experiments are compared, there is no striking apparent advantage for either method of processing with respect to vitamin content. However, the general quality of the precooked frozen puree *(c)* samples was markedly below that of the blanched frozen samples *(a)*. Bacterial counts were also made on these same samples by the methods and with media to be described in a subsequent section of this report. The original unprocessed puree was plated out and then the canned and frozen (both precooked and only blanched) were plated out after storage for five months. No significant change in total numbers of organisms occurred except that the heat-processed sample contained very few viable organisms. The precooked frozen sample gave an average count of 200 organisms per gram and the blanched pureed frozen sample gave an average of approximately 400 per gram, whereas the original sample had contained 150 per gram.

Bacteriological Problems and Keeping Qualities of Frozen Purees

In any discussions of plans for the commercial production of frozen purees for use in infant or invalid feeding, the question of survival of microorganisms in these products arises. As Tressler *(26)* pointed out, bacteria, as well as enzymes and vitamins, are a reliable index of quality in frozen foods. Very little data is available which could be applied specifically to this problem in purees. The early work reported by Magoon *(17)*, Berry *(3, 4, 5, 6)*, Wallace et al. *(29, 30)* and Tanner and Wallace *(25)* was concentrated mainly upon solving the problem of survival of microorganisms at low temperatures. All agreed that low temperatures killed many but not all of the molds, yeasts, and bacteria present in the food before it was frozen. Early studies (Haines, *10;* Prescott and Tanner, *21;* Straka and James, *23, 24;* and Prescott and Geer, *20*) also placed considerable stress upon the problem of survival of *Clostridium botulinum* in frozen foods, and if it survived, whether it might develop and produce toxin after thawing of the foods. All the investigators concluded that properly handled frozen vegetables posed no problem from this standpoint, but they all left doubt concerning what might happen if improper or careless handling should occur.

The problem has recently been reinvestigated thoroughly in a cooperative project of the Hooper Foundation of this University and the Western Regional Research Laboratory, U. S. Department of Agriculture. The conclusion has been drawn that there is no danger of *botulinus* toxin being present in frozen and thawed vegetables under any conditions. Acid producing bacteria generally develop first in frozen and thawed vegetables and these organisms have an inhibitory effect upon the growth and development of *Clostridium botulinum*.

Proctor and Phillips *(22)* made an extensive survey of the microorganism found by plating out samples of commercial frozen precooked foods. In meat and poultry products the predominant types were *Micrococcus* spp., in fish products *Flavobacter* spp., and in soups *Bacillus spp*. Many of the various products contained appreciable numbers of *Sarcina* and *Achromobacter* spp., *Staphylococcus* spp. were found in meat products, stews, poultry products and creamed products although in lower proportions than the other genera. Cooked foods experimentally inoculated with food poisoning strains of *Staphylococcus aureus,* alpha type *Streptococcus* and *Salmonella enteritidis* which were frozen and stored at 0° F., indicated that in some foods at least ten percent of the inoculated organisms survived such storage for six months. These types of bacterial pathogens in frozen precooked foods can increase in numbers after storage at defrosting temperatures of 30°-37° C. for six to eight hours. [See also Phillips and Proctor *(19)*].

While the results of these studies cannot be applied directly to frozen vegetable and other baby food purees, they are at least indicative of the possibilities and serve to emphasize the importance of using all possible means to control the entrance or development of such microorganisms.

Studies were made of the keeping quality of frozen purees after thawing and storing for a time both at room temperature (60°-75° F.) and in the household refrigerator (40°-42° F.) Vitamin C retention and bacterial plate counts on four kinds of agar were used as indices of keeping quality. The four agar media were eosine methylene blue (EMB), nutrient (N) made according to the standard formulae (American Public Health Association) *(1)*, yeast glucose (YG) and Wort (W) made as described by Vaughn *(27)*.

The vitamin assays and platings were made immediately after thawing, after storage at room temperature in the original container for one and two days and after storage in the refrigerator for one, two, three, and four days. No special precautions other than the usual care and cleanliness exercised in laboratory preparation of foods and cleaning of containers were taken either during preparation or storage of these samples. The platings and vitamin assays were discontinued after what was considered to be a reasonable maximum storage time after thawing, under the particular conditions of storage. These particular media were chosen for

platings because the combination would be likely to favor the growth of all types of organisms which might be found on these type of foods. Eosine methylene blue agar encourages the growth of lactose—utilizing Gram-negative organisms. In yeast glucose agar, lactic acid bacteria or yeasts (whichever is predominant in the population) are encouraged. Nutrient agar allows the development of aerobic spore formers, Gram-negative organisms, molds and yeasts to a slighter extent. Lactic acid bacteria are suppressed. Wort agar is particularly suitable for the growth of yeasts and molds. Tables 12 and 13 summarize the results of the platings of the samples held at these two temperatures after thawing. The organisms which were counted in these platings represent mainly or entirely the saprophytic organisms commonly present in vegetables and fruits. More data on these organisms as well as on the survival of the more dangerous pathogens or toxin producers would be desirable.

Table 14 gives a typical series of examples showing vitamin C retention of purees stored in a refrigerator for several days after thawing. It may be concluded from these results that this vitamin remains quite stable in these products after thawing except in some fruits, especially if they have been prepared without blanching.

TABLE 12

Summarized Results of Dilution Plate Counting of Purees Stored at Room Temperature (65-75° F.)

Kind of Puree	Storage time after thawing (days)	Numbers of organisms per gram			
		Medium			
		YG	N	W	EMB
Broccoli	0	2,200	2,200		675
Broccoli	1	11 x 10^6	16 x 10^6		12 x 10^6
Broccoli	2	200 x 10^6	155 x 10^6		167 x 10^6
Broccoli + Asparagus	0	1,900	1,900		440
Broccoli + Asparagus	1	49 x 10^6	16 x 10^6		23 x 10^6
Broccoli + Asparagus	2	540 x 10^6	275 x 10^6		130 x 10^6
Raw guava	0	20	18	7	
Raw guava	1	130	290	76	
Raw guava	2	700	405	55	
Steamed guava	0	8	20	7	
Steamed guava	1	985	1,045	640	
Steamed guava	2	105,000	56,000	81,000	
Persimmon	0	2,000	2,300	1,800	
Persimmon	1	3,000,000	3,950	3,000,000	
Persimmon	2	.....*	.....*	.....*	
Prune (dried)	0		3	0	
Prune (dried)	1	14	28	6	
Prune (dried)	2	72	206	10	

* Not plated because visibly fermenting.

TABLE 13

Summarized Results of Plate Counting of Purees Stored in the Refrigerator (40-42° F.)

Sample	Storage time after thawing (days)	Numbers of organisms per gram			
		Medium			
		YG	N	W	EMB
Broccoli	0	2,300	3,600		700
Broccoli	2	2,300	2,900		400
Broccoli	4	23,000	20,000		2,000
Broccoli + Asparagus	0	1,600	2,000		750
Broccoli + Asparagus	2	2,100	3,400		700
Broccoli + Asparagus	4	4,500	6,000		600
Raw guava	0	50	41	10	
Raw guava	2	185	155	120	
Raw guava	4	170	380	135	
Steamed guava	0	14	17	15	
Steamed guava	2	6	11	7	
Steamed guava	4	14	25	14	
Persimmon	0	1,800	2,200	1,800	
Persimmon	2	4,600	3,900	3,300	
Persimmon	4	70,000	80,000	470,000	
Prune (puree of dried)	0	4	12	2	
Prune (puree of dried)	2	3	12	2	
Prune (puree of dried)	4	12	19	9	

TABLE 14

Ascorbic Acid Retention in Frozen Purees Stored in a Household Refrigerator After Thawing

Sample and Treatment	Vit. C 0 hrs. after thawing mg./ 100 gm.	Vit. C 24 hrs. after thawing mg./ 100 gm.	Vit. C 72 hrs. after thawing mg./ 100 gm.	Percent retained during 72 hrs. at 40° F.
Broccoli Puree	56.6	56.4	54.7	96
Pea Puree	25.1	21.8	22.0	87
Peach + Apricot Puree	4.9	2.5	....	....
Peach + Apricot Puree (another sample)	2.8	1.8	1.2	43
Raw Muir Peach Puree	3.2	2.1	1.4	43
Peas + Pods Puree	24.7	20.8	17.2	69
Broccoli + Asparagus	44.8	39.8	38.7	86
Broccoli + Asparagus (another sample)	32.5	30.5	28.8	88
Blanched Peach + Nectarine Puree	11.4	....	10.5	92
Raw Peach + Nectarine Puree	6.4	3.1	2.2	34
Raw Guava Puree	179.0	169*	165	92
Steamed Guava Puree	173.0	172*	167	97

* 48 hrs.

Acceptability of Frozen Compared with Canned Purees

In order to test the relative acceptability to invalids of frozen and canned purees, some preliminary tests were made on samples of mixed green vegetables and vegetables plus beef as put up by a commercial baby food firm. The samples herein designated as "canned" were processed in a retort in the usual manner, while those designated as "frozen" were taken from the same cannery line after filling and sealing the cans but before heat processing. These samples were then "quick frozen" at — 20° F., in a nearby commercial air-blast freezing tunnel. This procedure had the advantage of allowing uniformity of original material. Its disadvantage was that the frozen vegetables were precooked more than they would ordinarily be, so that the color of the green vegetables was not the bright green characteristic of the frozen green vegetable purees which we prepared on a laboratory scale, or which can also be prepared commercially. A sample of commercially frozen asparagus puree was also tested. Canned baby food manufacturers do not include asparagus puree among their selections, thus making it impossible for us to test a parallel canned sample. These samples were then distributed to two hospitals allowing fifty cans of each kind of food for each hospital. The dietitians who cooperated, heated and served the respective canned and frozen samples side by side to patients who were on soft diet and to a few members of the hospital staffs and kitchen personnel. The tasters were asked to answer the following questionnaire:

SCORE SHEET FOR TWO SAMPLES OF PUREES

(Check one) (a) (b)

1. I definitely like the sample
   Acceptable, but not outstanding
   I definitely dislike the sample
2. *Flavor*
   Good
   Fair. Not outstanding
   Poor
3. *Texture*
   Good firm feel
   Too thin and watery
   Too thick

4. *Color*
   I like the color
   Color is not objectionable
   I dislike the color

If the patients were very ill, the nurses or dietitian filled in the questionnaires. In some cases they were too ill to discriminate or remember what they had eaten. This probably accounted for the very large proportion of the tasters who expressed no preference. The data are summarized in Table 15. The interesting points brought forth from this study are: (1) There was a strong preference for the frozen samples of both types

TABLE 15

Summary of results of comparative acceptability of frozen and canned purees in hospital tests

(All figures represent numbers of individual tasters)

	Mixed Green Vegetables	Vegetables + Beef
Preferred canned sample	9	11
Preferred frozen	32	41
Expressed no preference	19	33
Preferred flavor of canned sample	9	13
Preferred flavor of frozen sample	34	38
Expressed no preference for flavor	20	34
Preferred texture of canned sample	0	10
Preferred texture of frozen sample	12	17
Expressed no preference for texture	48	57
Preferred color of canned sample	2	4
Preferred color of frozen sample	6	13
Expressed no preference for color	52	67

of puree, mainly on the basis of flavor. (2) Texture or consistency of the puree, which we consider to be one of the outstanding disadvantages of the frozen product, made little difference to these individuals. (3) The difference between the frozen and canned samples with respect to color was not of noticeable importance to them. However, as mentioned earlier, it was not as striking in these samples as it would have been if the frozen samples had received less heat treatment prior to freezing.

Packaging

A problem which was not studied systematically by us, but which is of extreme importance to commercial production of such a product is that of packaging. Most of our experimental samples have been frozen in either tin cans, screw capped or crown capped glass jars, cellophane-lined paper cartons, or heavily waxed paper cartons with push-in lids (Nestrite type). These have all been satisfactory for experimental purposes and one or more might readily be adapted for commercial use. Small enough amounts for one or two servings for an infant might also be packaged individually in plastic sausage casings or in small cellophane containers about the size of ice cubes which might then be grouped into larger packages offering a variety of foods in a single dispensing unit.

Summary and Conclusions

A report of the work which has been completed to date on the experimental preparation and testing of frozen fruit and vegetable purees is given.

The results of a survey on regulations and standards pertaining to the canned baby food industry revealed that while there are no special regulations intended for this specific purpose, high standards are maintained as a result of consumer demand and industry competition. Commercially canned baby foods were chemically analyzed and the results were compared with analyses of experimentally frozen samples.

Several methods of preparing fruit and vegetable purees for freezing were tested and the results compared. The best results with fruit purees were obtained by blanching the fruit just long enough to inactivate the enzymes that catalyze darkening and then preparing the puree. For some fruits partial dehydration prior to pureeing was successful. In the preparation of vegetable purees, blanching as for regular freezing, followed by pureeing and freezing, yielded the best quality. Cooking had to be completed at the time of serving. This was also advantageous from the microbiological point of view. The use of several varieties of fruit, antioxidants added to fruit purees, and vitamin retention during storage were all included in the studies.

A survey of the literature indicated that there would be slight danger of pathogenic organisms surviving freezing storage. However, a direct study of this factor in relation to fruit and vegetable purees is needed. A few data were presented indicating that after thawing, these purees may be kept bacteriologically and nutritionally in good condition in a household refrigerator for two or three days. At room temperature they should not be held longer than one day after thawing.

A preliminary survey was made of acceptability to hospital patients of frozen compared with canned purees. There was a definite trend toward preference of the frozen for flavor and of the green vegetables for color. Additional data along these lines are required before definite conclusions can be drawn.

Further data on packaging, acceptability, bacteriology and some aspects of preparation, especially with a view toward improving consistency, are required. However, the general conclusion which might be drawn is that frozen purees should be capable of commercial production and consumer acceptance, in order to supplement and add variety to, rather than compete with, the familiar canned products, and to expand the market for fresh produce.

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